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OENOTHERA GIGAS NANELLA, A MENDELIAN MUTANT

HUGO DEVRIES

In a recent book GATES has studied the significance of the experiments made with species of *Oenothera* as proofs for the general theory of mutation, and has given an exhaustive and critical review of the facts in this rapidly increasing field of research.[1] He has laid special stress upon the results of crosses, which show the great diversity of these phenomena when studied in some wild plants, as contrasted with the now prevailing doctrine of Mendelism; for among the mutants of *Oenothera* instances of Mendelism are rare. The first known example is that afforded by *O. brevistylis*, which follows the law of MENDEL as a recessive in all its crosses with the parent species, with other mutants, and with other species of the same group.[2] But, unfortunately, the production of this form by means of mutation from *O. Lamarckiana* is so rare that it has not, as yet, been repeated under experimental control. Another instance is *O. rubricalyx*, discovered and studied by GATES (*op. cit.*, p. 103), which behaves as a dominant in its crosses with *O. Lamarckiana*.

In this article I hope to show that the dwarf character, which in so many instances complies with the formulae of MENDEL, but which behaves in a different way in crosses of the derivatives of *O. Lamarckiana*, may, at least in one instance in this group, follow that law as exactly as in any other pure Mendelian case. This

[1] GATES, R. R., The mutation factor in evolution. London. 1915.

[2] DEVRIES, HUGO, Die Mutations-Theorie. 1:223; 2:151–179, 429.

instance, therefore, affords a means for the experimental study of the origin of such a form by mutation. The main result of this study is the proof of the occurrence of mutant Mendelian hybrids besides the pure dwarfs.

In my book on the mutation theory I have pointed out that the origin of *O. brevistylis* from *O. Lamarckiana* may have been induced by the mutation of a single sexual cell. If this combined in fertilization with a normal gamete, a hybrid would be produced which would not be distinct from the parent species in its external features. This hybrid would then, in its self-fertilized seeds, follow the law of MENDEL and produce, besides constant *Lamarckiana* plants, partly hybrids of the same type and partly specimens of the type of *O. brevistylis*. From this origin and the subsequent free intercrossing in the field, the yearly appearance of *O. brevistylis* would receive a sufficient explanation (DEVRIES, *op. cit.*, p. 506).

If the process of mutation into this type were more often repeated, it should be possible to discover the original hybrids. They would, it is true, not be discernible from their normal sisters by external marks, but would yield, after artificial self-fertilization, about 25 per cent of *brevistylis*. And since mutants are produced ordinarily in a proportion of 1–2 per cent or less, the difference would be large enough to be noticed. Until now, however, such cases have not been observed.

I have, therefore, been looking for another example in which a Mendelian behavior of the mutants might be associated with a normal coefficient of mutation from the parent species. Such cases would betray themselves by exceptionally high coefficients in single parent plants. Instances of such individual deviations are very rare, partly on account of the necessarily limited number of mother plants from which the seeds of our cultures are taken. But SCHOUTEN[3] has observed that *Oenothera gigas*, which ordinarily produces 1–2 per cent dwarfs, may be seen to throw them off in as large a number as 15 per cent. The same phenomenon has been described by GATES (*op. cit.*, p. 137), who counted 9 per cent and 11 per cent of dwarfs among the offspring of two self-fertilized plants of *O. gigas*.

[3] SCHOUTEN, A. R., Mutabiliteit en Variabiliteit. 1908.

From time to time I have noticed the same deviating percentages in my own cultures. Thus, for instance, I fertilized in 1910 a specimen of *O. gigas* by its own pollen, and among 50 seedlings of its offspring 10 were dwarfs, pointing to a percentage of about 20 per cent.[4] Similar facts have since occurred more than once in my cultures.

SCHOUTEN and GATES have interpreted these figures as indicating a Mendelian proportion of dwarfs, and on this assumption the parent plant would have been a mutant hybrid in the same sense as explained above for *O. brevistylis*. Mutant hybrids would then occur in a race which produces dwarf mutants also, and the latter would then, of course, have to be considered as the products of the combination in fertilization of two sexual cells, both of which had mutated into *nanella*. The production of dwarfs from *O. gigas* would then follow the same process which is to be assumed for the origin of *O. gigas* itself from *O. Lamarckiana;* and the copulation of two similarly mutated cells would then more easily be accessible to experimental investigation.

In order to verify the exactness of this conception I have followed up the progeny of such a presumed mutant hybrid, and on the other hand have made crosses between *O. gigas* and *O. gigas nanella*. In both cases the truth of the assumption was easily ascertained.

Mutations of single gametes may be discovered by different means in other instances also, the production of potential *nanella* gametes by *O. Lamarckiana* being the most likely to be betrayed in this way.[5] I have observed such cases in crosses between *O. Lamarckiana* and *O. rubrinervis*. From these ordinarily two types arise in the first generation, one of which resembles the mother and the other the father. In my book on *Gruppenweise Artbildung* I have called them "*Lamarckiana*" and "*subrobusta*." Both types are usually constant after self-fertilization. But, from time to time, individuals appear which in their progeny produce an unexpected number of dwarfs. The following cases may be adduced.

[4] DEVRIES, HUGO, Gruppenweise Artbildung. p. 340. 1913.

[5] Besides the production of gametes for *gigas* by *O. Lamarckiana*, as shown by the occurrence of specimens of *semigigas* in self-fertilized strains of the parent species, or by the production of the Hero-type in crosses of *O. Lamarckiana* with allied species.

The *rubrinervis* strain for these experiments had arisen as a mutant from *O. Lamarckiana* in 1895, and its second generation was cultivated in 1905. No dwarfs were produced in the first generation after the crosses, and in the second only from single individuals, the remainder giving either no dwarfs at all or only about 1 per cent, by ordinary mutation.

TABLE I

EXCEPTIONAL PRODUCTION OF DWARFS BY SINGLE PLANTS OF *Oenothera subrobusta*

Cross	Cross	1 Gen.	2 Gen.	Number of individuals	Percentage of nanella
Lamarckiana×rubrinervis....	1905	1913	1914	140	9
rubrinervis×Lamarckiana.....	1905	1907	1913	70	11
rubrinervis×Lamarckiana.....	1907	1913	1914	70	16

If we compare these figures with the results of the crosses between *O. rubrinervis* and *O. nanella* itself, as described in my *Gruppenweise Artbildung* (p. 215), we find a complete analogy, since these crosses give no dwarfs in the first generation, and in the second about 10–14 per cent from the self-fertilized specimens of *O. subrobusta*. It is evident, therefore, that the exceptionally high yield of dwarfs in these crosses of *O. Lamarckiana* and *O. rubrinervis* must be the product of latent mutations which occurred in some of the sexual cells of one of the parents. And since *O. Lamarckiana* is known to produce ordinarily 1–2 per cent dwarfs, while *O. rubrinervis* does not show signs of such a mutability, we may confidently assume that our figures indicate latent mutations of sexual cells of *O. Lamarckiana*.

BARTLETT[6] recently described a similar instance of an unexpectedly high mutability, and proposed for it the same explanation, on the assumption of a latent mutation of a sexual cell in a previous generation. This case is of the greatest interest since it relates to a pure species and not to the discovery of mutated gametes by means of crosses as in the experiments just described. The mutating species was *O. Reynoldsii* Bartlett, one of the forms of the old *O. biennis*. It produced in 1913 three marked types, one repeating the parental form, and the two others being dwarfs and called

[6] BARTLETT, H. H., Mutation en masse. Amer. Nat. 1915.

mut. *semialta* and mut. *debilis*. The latter is, on the average, about half as high as the former. This curious segregation repeated itself in the next generation in 1914, not from all the individuals, but from only one of the two whose offspring have been tried in this respect.

Similar proofs of latent mutations of sexual cells may evidently be expected to occur in other strains also and will have to be looked for in all cases of an unexpectedly high degree of mutability.

I will now return to my experiments on the production of dwarfs by *O. gigas*. In order to obtain specimens of *O. gigas* yielding a high percentage of dwarfs from their seeds, I sowed in 1911 seeds of my pure strain, cultivated the plants as biennials, and fertilized them in 1912 by their own pollen, in bags. They were vigorous plants of the fourth generation (*Gruppenweise Artbildung*, p.175), and yielded a large harvest of seed, which was sown in 1913, and served as a criterion, since no essential differences were to be seen on the plants themselves. Moreover, I used the seeds of some good biennial specimens of the previous or third generation. The ancestors of all these plants had been fertilized by myself in bags down from the mutant in 1896 which started the race. The harvest of 1912 and 1910, sown in 1913, gave the result as shown in table II.

TABLE II

A. PERCENTAGES OF DWARFS AMONG OFFSPRING OF *O. gigas*

Generation	Number of seed-bearer	Total of seedlings	Dwarfs	Percentage of dwarfs
4th generation.....	1	174	0	0.0
"	2	176	1	0.6
"	3	191	34	17.8
"	4	154	1	0.6
"	5	166	1	0.6
3rd generation.....	6	164	0	0.0
"	7	43	1	2.3
"	8	52	0	0.0
"	9	132	2	1.5
"	10	130	0	0.0

From a second strain, derived from the same mutant and described in my *Gruppenweise Artbildung* (p. 175), I had in 1911–1912 nine biennial specimens which yielded a sufficient harvest.

Tried in the same way, they gave the percentages of dwarfs shown in table III.

TABLE III

B. DWARFS OF *O. gigas*

Generation	Number of seed-bearer	Total of seedlings	Dwarfs	Percentage of dwarfs
6th generation.....	1	132	0	0.0
"	2	165	0	0.0
"	3	155	1	0.6
"	4	161	0	0.0
"	5	159	25	15.7
"	6	76	0	0.0
"	7	151	0	0.0
"	8	130	0	0.0
"	9	124	19	15.0

All in all, 19 specimens were studied. Among them three gave a percentage of 15–15.7–17.8, but the others gave only 1–2 per cent or no dwarfs at all. The dwarfs produced by this latter group were evidently due to ordinary mutability, but the figures for the former group differed too widely from these to be looked at in the same way. I consider them to be due to Mendelian segregation, and assume that the fact that they fall short of the expected 25 per cent is due to the difficulties of cultivation and to a less viability of the dwarfs as compared with the normal specimens.[7] I chose no. 3 of the first group (17.8 per cent dwarfs) for continuing the experiment.

If the segregation in this second generation followed the law of MENDEL, then among the plants of normal stature one-third must be constant in their progeny and the remainder must split up according to the same law. I succeeded in having a dozen of plants flower and ripen their seeds as annuals, fertilized them purely, and sowed the harvest in the spring of 1914. The result is given in table IV.

Three of the individuals yielded no more dwarfs than in ordinary mutation, and the seven others showed figures which approach the Mendelian law as nearly as might be expected. If we combine these figures with the 17.8 per cent of dwarfs of the former generation, we find for this about 18 per cent dwarfs, 57 per cent hybrids

[7] See GATES, *op. cit.*, p. 89.

of high stature, and 25 per cent normal high specimens. This may be considered as sufficient proof that the splitting took place after the law of MENDEL.

TABLE IV

C. DWARFS AMONG THE OFFSPRING OF *O. gigas* (A, no. 3)

Number of seed-bearer	Total of seedlings	Dwarfs	Percentage of dwarfs
1	242	1	0.5
2	276	0	0.0
3	177	1	0.5
4	237	39	16.0
5	238	52	22.0
6	236	50	21.0
7	196	42	21.0
8	81	25	31.0
9	269	59	22.0
10	265	57	21.0

The dwarfs were counted in June and July, and the degree of development at this time corresponded with the photographs given in my *Gruppenweise Artbildung*, p. 316, *figs. 115* and *116*. At this period they are clearly distinct from the normal specimens and so there was no difficulty in counting them. In some specimens of *O. gigas* mut. *nanella* the number of chromosomes has been determined and was found to be the same as in *O. gigas* itself (28), as was to be expected. Partly on account of this fact, partly in consequence of the nearer relationship, the fecundations did not experience the difficulties which are connected with crosses between *O. gigas* and *O. Lamarckiana* mut. *nanella*. They succeeded fairly well and yielded, as we have seen, relatively large numbers of seeds.

The Mendelian behavior of the production of dwarfs by means of mutation from *O. gigas*, moreover, may be proved in another way. If the mutant hybrids of this form are fertilized by the pollen of *O. gigas nanella*, the expectation will, of course, be the production of 50 per cent of tall specimens and 50 per cent of dwarfs. But, on account of the smaller viability of the latter, we should have to be content with somewhat smaller numbers. In 1913, therefore, I crossed some specimens of apparently normal *O. gigas* with the pollen of a constant race of *O. gigas nanella*, my culture being the third generation derived from a mutant of 1910 (*Gruppenweise Artbildung*, pp. 315–316). I was fortunate in choosing, among

some normal plants, two mutant hybrids, and will give the constitution of their progeny, together with that of two normal individuals of *O. gigas*, in table V. The numbers of seedlings have been very small in this case, owing to the small degree of fertility of the pollen of *O. gigas nanella*.

TABLE V

DWARFS IN THE FIRST GENERATION OF *O. gigas*×*O. gigas nanella*

Number of seed-bearer	Number of seedlings	Dwarfs	Percentage of dwarfs
1	38	11	30
2	65	28	43
3	28	1	3
4	59	2	

The first two seed-bearers had evidently about one-half of their egg cells mutated into *nanella*, which by the fertilization with the pollen of dwarfs must, all of them, become *nanella* specimens. The two last-named plants, although externally not differing from the others, had only very few mutated sexual cells, and therefore produced only about 3 per cent of dwarfs.

TABLE VI

DWARFS IN THE SECOND GENERATION OF *O. gigas*×*O. gigas* MUT. *nanella*

Seed-bearer	Total of seedlings	Dwarfs	Percentage of dwarfs
A. O. gigas nanella×O. gigas			
No. 1	291	45	15
No. 2	69	12	17
B. O. gigas×O. gigas nanella			
No. 1	60	16	27
No. 2	310	73	24
No. 3	304	62	20
No. 4	74	14	19
No. 5	283	46	16
No. 6	210	30	14
C. O. gigas mut. hybrid×O. gigas nanella	326	52	16

The experiment showed at the same time that hybrids between *O. gigas* and *O. gigas nanella* have the features and the stature of the former type, and thereby justified the assumption made above in the explanation of the behavior of mutant hybrids.

I made the reciprocal cross in the same year, fertilizing some dwarfs of my race by the pollen of normal plants of *O. gigas*. The

fecundation was a difficult one and I got only 38 seedlings, all of which developed into tall plants of the stature and character of *O. gigas* (1914).

In order to study the segregation of dwarfs in the next generation I fecundated a number of specimens of the three described groups of artificial hybrids and sowed their seed in 1915. On the basis of Mendel's law the expectation is, for all of them, 25 per cent dwarfs, or somewhat smaller numbers on account of the lesser viability of these dwarfs. The sowings of 1915, counted in May and June, gave the results shown in table VI.

These figures give sufficient proof that the crosses between *O. gigas* and its dwarfs follow the law of MENDEL.

Summary

1. *Oenothera gigas* produces dwarfs (about 1–2 per cent) and mutant hybrids of normal stature, which after self-fertilization give 15–18 per cent, theoretically 25 per cent, of dwarfs.

2. These mutant hybrids split up, after self-fertilization, according to the law of MENDEL, yielding about 18 per cent dwarfs, 25 per cent normal specimens of tall stature, and 57 per cent hybrids of the same type. The latter gave about 21 per cent of dwarfs among their progeny.

3. The mutant hybrids, fertilized by *O. gigas nanella*, yield 30–43 per cent, theoretically 50 per cent, of dwarfs.

4. In artificial crosses with *O. gigas* the dwarfs follow the law of MENDEL.

5. The production of dwarfs from *O. gigas* by means of mutation, therefore, is to be considered as requiring the copulation of two gametes, both of which are potentially mutated into dwarfs. The mutant hybrids must then be the result of the fertilization of a mutated gamete by a normal one. They are correspondingly less rare than the dwarfs themselves.

6. In combination with the fact that the dwarfs of *O. Lamarckiana* do not follow the law of MENDEL, either in their origin by mutation or in artificial crosses with the parent species, these conclusions reveal a new differential character between *O. gigas* and its parent species.

AMSTERDAM

THE INFLUENCE OF THE TANNIN CONTENT OF THE HOST PLANT ON ENDOTHIA PARASITICA AND RELATED SPECIES[1]

MELVILLE THURSTON COOK AND GUY WEST WILSON

The differences between species, genera, and families by which certain groups of plants are resistant to certain parasites while others are more or less susceptible are very generally recognized by all botanists. *Actinomyces scabies* attacks potatoes, turnips, beets, carrots, and parsnips, representing four different families. *Pseudomonas tumefaciens* attacks a much larger range of host plants. Many familiar parasitic fungi and bacteria are restricted to certain families, to a few genera, or even to a single genus; others have similar restrictions as regards species; and still others are restricted to the races within the species. The same law will apply to the insects which cause the peculiar physiological or pathological structures known as galls. *Cecidomyia pilulae* occurs on a very large number of oaks, *Amphibolips confluentus* on four species, while the very common *Andricus seminator* occurs on but one.

Individual plants which are more or less immune to destructive parasitic organisms have attracted the attention of the plant pathologists and plant breeders and have been the starting point for long series of selection experiments resulting in resistant varieties which are of great value to the practical agriculturists. Many theoretical explanations of the resistance or susceptibility of related varieties or species have been offered by botanists who should know better, but very few facts of real value have been collected.

MARSHALL-WARD'S studies indicated that the histological characters were of but little if any importance in aiding the plant to resist its parasitic enemies. He finally says: "Infection and resistance to infection depend on the power of the fungus protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins; and reciprocally, on that of the protoplasm of

[1] A more complete discussion of the experiments given in this paper will appear in some of the publications of the New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

the cells of the host to form auto-bodies which destroy such enzymes or toxins, to excrete chemotactic substances which expel or attract the fungus protoplasm."[2]

The little work that has been done indicates that these problems involve both plant pathology and plant physiology, and that the plant pathologist must give more and more attention to the fundamental problems in plant physiology.

Some years ago the senior author and Dr. J. J. TAUBENHAUS conducted a series of experiments on the relation of parasitic fungi to the cell contents of the host plants.[3] The primary object of this work was to determine to what extent tannin might be a factor in enabling the host plant to resist parasitic fungi. Although the fungi which attack fruits were used for most of these experiments, some attention was given to *Endothia parasitica*. This organism gave a good growth of mycelium and scant spore formation when grown on an agar medium containing 0.6 per cent tannin, but a less toleration to tannin when grown on a liquid medium. It was also evident that its toleration of tannin was somewhat dependent on the character of the food supply.

A little later, CLINTON[4] made similar experiments with *Endothia*, using commercial tannin (M. C. E. Brand, U.S.P.) in amounts varying from 0.2 per cent to 14 per cent. All cultures grew in media containing as high as 4 per cent tannin; about one-half of the cultures of *E. gyrosa* grew on media containing 8 per cent tannin, but all failed to grow on cultures containing more than 12 per cent. *E. parasitica* was a little more tolerant to tannin than the other species used. CLINTON used a potato agar, and therefore his results are not comparable to those obtained by COOK and TAUBENHAUS, who used synthetic media.

Although the results of all of these studies indicate that tannin is in a measure toxic to fungi, the report of the chemist of the

[2] MARSHALL-WARD, H., Recent researches on parasitic fungi. Ann. Botany 19:1–54. 1905.

[3] COOK, MEL. T., and TAUBENHAUS, J. J., Relation of parasitic fungi to the cell contents of the host plant. 1. Toxicity of tannin. Delaware Agric. Exp. Sta. Bull. 91. pp. 77. *figs. 43.* 1911.

[4] CLINTON, G. P., Chestnut bark disease, *Endothia gyrosa* var. *parasitica* (Murr) Clint. Ann. Report Conn. State Agric. Exp. Sta. 1912. pp. 359–453. *pls. 21–28.* 1913.

Pennsylvania chestnut tree blight disease commission indicated that the tannin content of the diseased bark was higher than that of the healthy bark. This report appears to contradict the idea that tannin in the bark is toxic to the fungus, otherwise the fungus would be destroyed by the tannin. However, this point was also investigated by KERR, who says "the increment of tannin is only apparent and does not really occur. We have found all decayed wood and bark give higher tannin contents, no matter what causes the decay. It simply means that other constituents have decomposed and disappeared, while the tannin remains practically stable."

It is very evident to the writers that commercial tannin is a very uncertain substance, as packages of tannin from the same manufacturers and supposed to be the same were found to give different results when used in cultures. It was also evident that ordinary methods of determining tannin are unsatisfactory. Therefore, KERR, a well known technological chemist of Lynchburg, Virginia, who has devoted considerable attention to the study of tannin, was asked to cooperate in this work. He furnished us with three extracts ("1–X," "2–X," "3–X," and "A") which are described in connection with the experiments. In addition to these extracts we also used commercial tannin (MERCK) for comparison.

Source of cultures

Cultures of various American species of *Endothia*, as well as foreign strains of some of the species, were obtained from various laboratories. In addition, some strains of *E. parasitica* were isolated in our own laboratory. We have indicated these by the names and the serial numbers used by the laboratories when they came to us, except in the case of *E. parasitica*. In this instance we have uniformly referred to this fungus as *E. parasitica*, without regard to whether it was considered a species or a sub-species at the source of supply.

The use of the specific names of *E. gyrosa* and *E. radicalis* varies in different laboratories, according to SHEAR and STEVENS.[5] In the light of this paper it appears that the fungi from CLINTON

[5] SHEAR, C. L., and STEVENS, F. E., Cultural characters of the chestnut blight fungus and its near relatives. U.S. Dept. Agric. Bur. Pl. Ind. Circ. 131. pp. 18. 1913.

labeled *E. gyrosa* and that from STEVENS called *E. radicalis* are in reality identical. Our culture work also leads us to the same conclusion. Therefore, in order to avoid confusion, we will use the name *E. radicalis*, but will in such case indicate the origin of our original culture.

A careful study of our cultures indicates that we have only three distinct species: *Endothia parasitica* (American and Chinese races), *E. radicalis* (*E. gyrosa* and *E. virginiana*), and *E. radicalis mississippiensis*.

Several months were devoted to preliminary work to determine the most desirable medium, best methods for mixing the tannin into the medium, and for perfecting the technique of the work. The formula for the most satisfactory medium, the one which was used in all our work, is as follows: water 1000 cc., glucose 20 grains, peptone 10 grains, potassium phosphate (monobasic) 0.25 grain, magnesium sulphate 0.25 grain. A given series of cultures was always made from the same lot of medium, treated with the same extract, inoculated in the same manner, and kept under exactly the same conditions.

The difficulties arising from the use of tannin in a medium containing proteid were not overcome. The first difficulty encountered is the fact that commercial tannin (MERCK) is an unstable and variable substance. According to FISCHER (Ber. Deutsch. Chem. Gesells. **36**:3252. 1913), tannin is an anhydrous glucoside of gallic acid. This relationship makes it easily convertible by hydrolysis into gallic acid and related substances. *It is therefore entirely possible that no sterile culture medium can be prepared which contains all the tannin unchanged.*

The usual statement that tannin in contact with proteid forms an insoluble precipitate has not been borne out by our work. Indeed, comparatively large quantities of tannin may be added to the agar formula which we used without changing perceptibly either the tannin or the proteid, so far at least as we were able to determine.

The experiments were conducted with two lots of MERCK'S tannin. The first of these was already in stock at the time the work was undertaken. By using a 10 per cent solution of this tannin, as much as 2 per cent of tannic acid could be added to the

agar without changing the composition of either. However, to accomplish this it was necessary to allow the tannin solution to run slowly from a pipette into the melted agar while the latter was constantly agitated. If the tannin was added too rapidly, or the agitation of the agar was insufficient, more or less coagulation resulted. With a 20 per cent aqueous solution of tannic acid, less than half this amount (0.8 per cent) could be added without coagulation. Moreover, even a very small amount of tannic acid in its solid form would cause coagulation in the agar. Another lot of MERCK'S tannin was of such character that only about half as much tannic acid could be added to the agar without change.

When first placed in the agar, the tannic acid caused a milky appearance, which disappeared on sterilization. Where high percentages of tannin were used, the agar upon sterilization showed a tendency to become viscid (about 0.8–2 per cent) or even liquid (about 2–2.5 per cent). The transition between viscosity and liquefaction is gradual in such a series as we used, where each member differed from the next by 0.2 per cent of tannic acid. In no case was the distinct curd which various investigators have described to be observed. In agar with 3 per cent of tannic acid the entire mass of medium becomes a clear liquid with a thin film of solid matter on one side of the test tube if set for a slant, or in the bottom if set upright. This solid material gives the same reaction both to Millon's proteid test and to the ammonium molybdate test for tannic acid as does the solid agar of the lower members of the series. Similar results were obtained by testing the liquid portion of the medium. Evidently, the explanation of this liquefaction is to be found in some other direction than the chemical interaction of tannin and proteid.

If the agar medium used is titrated to various degrees of acidity and a series of such tubes sterilized, it is found that the agar ranges from solid through viscid to liquid; that is, the same phenomenon can be induced by acidulating the medium as by the addition of the tannic acid. In each case a more careful test of the nature of the proteid substance in the liquid from the acidulated agar shows that proteid digestion has progressed so far that the power of solidification has been lost.

These considerations naturally raise the question of the acidity of the culture medium containing tannic acid. Our tests showed that a 3 per cent aqueous solution of tannic acid is about +65 Fuller's scale. This is considerably higher than is indicated by CLINTON (Report Conn. Agric. Exp. Sta. 1912. p. 432). However, as CLINTON used a vegetable (potato agar) medium, while we used a synthetic medium, the results are in no wise comparable, as no account is taken by CLINTON of the effects on the tannic acid of the various organic constituents of the medium to which he added it.

Gallic acid was similarly tested, but failed to show any coagulating effect either on the agar medium or on its constituents.

The various materials furnished by KERR behave in much the same way toward agar as does commercial tannin. His purer tannin extracts, however, do not liquefy the agar at as low percentages as does commercial tannin. Those extracts from which the coloring matter had not been removed had a more pronounced action on the culture medium than even the second lot of MERCK'S tannin. The original acidity of the agar and the quantity and nature of the impurities which may be present in the tannin appear to modify to a great extent the chemical activities upon the admixture of the two substances in completing the culture medium.

To test tannin fermentation

To test the ability of the fungi to live in pure solutions of tannin and related substances, two experiments were made with varying strength of aqueous solutions of these materials. In the first series, the spores were sown on the medium, and after germination cubes about 5 mm. in size were transferred to the tannin solution. In these tests *E. parasitica* and the European strain of *E. radicalis* were used.

The first test included MERCK'S tannin and KERR'S extracts "A" and "1–X," of 2.05 per cent and 5 per cent solution. The strength of the solution appeared to have less effect on the fungus than did the nature of the material. The two species of fungi showed no more difference in quantity and vigor of growth than would be expected for two strains of the same species.

On tannin neither species grew even as much as might have been expected from the nutriment stored in the agar block.

On extract "A" a fair growth was made, using up the food material stored in the original agar block and forming masses of mycelium about 2 cm. across and producing abundant pycnospores.

On extract "I–X" an abundant growth was secured, entirely filling the liquid in the flask with a thick growth of mycelium which rose above the liquid and produced abundant pycnospores.

The second series was made up in strength of 0.2, 0.4, 0.6, 0.8, and 1 per cent tannic and gallic acid. These were sown with spores of the same fungi used before. In no case was growth made, although check sowing on agar showed the spores to be viable.

Endothia on tannin

GROWTH OF ENDOTHIA ON COMMERCIAL TANNIN (MERCK)

The cultures used for this series were made to contain 0.1 per cent, 0.2 per cent, 0.4 per cent, and by intervals of 0.2 per cent up to 2.4 per cent of tannin. In this series of experiments the agar remained firm, except in one or two of the cultures containing the highest percentages of tannin, in which there was a slight tendency to a semifluid condition. No cultures showed sufficient proteid digestion to allow the formation of a liquid. The following strains of *Endothia* were used: *E. parasitica* (STEVENS no. 1158), a Chinese strain from the Bureau of Plant Industry,[6] and a strain designated "P.P." of our own isolation from Prospect Park (Brooklyn) material; *E. radicalis* (METCALF no. A, STEVENS no. 2391, a European strain secured from ANDERSON, and CLINTON'S *E. gyrosa* no. 7677); and *E. radicalis mississippiensis* (STEVENS nos. 1196 and 3443).

Endothia parasitica (American strain) gave a good growth of aerial mycelium, varying in amount in direct ratio to the percentage of tannin used. Poor growth on check until the end of the second week. Yellow color in mycelium at end of first week and discoloration of the agar during the second week. Cultures originally containing 0.2 gm. tannin (2 per cent) were sent to KERR,

[6] This culture was made from material sent directly from China to the Bureau of Plant Industry.

who was unable to detect any positive trace of tannin, but found a trace of gallic acid not exceeding 0.002 gm. This appears to to indicate that the fungus can use the tannin as a food. Pycnospores appeared on the check at the end of the sixth week, on 0.1 per cent tannin at the end of the seventh, on 1.4 per cent tannin at the end of the eighth, and ultimately on the 4 per cent tannin.

E. parasitica (Chinese strain) grew about the same as the American strain except that the growth was 5 days earlier and a lighter gray color. Pycnospores appeared during the fifth week on 2.4 per cent tannin and ultimately on the entire series.

It is rather remarkable that the Chinese strain is more tolerant to tannin than the American strain, and it raises the question whether this resistance is due to origin, age, or modification of the fungus since its first introduction into America, or some other cause.

E. radicalis (European) was more resistant than the American strain. Pycnospores were produced on 1.2 per cent tannin after 8 weeks' growth.

E. radicalis (American) gave an abundant growth of aerial mycelium, varying in color from yellow in the lower percentage of tannin to ashen in the higher. The presence of 0.2 per cent tannin was stimulating, but 0.8 per cent retarded the growth. No pycnospores were produced.

E. radicalis (CLINTON'S *E. gyrosa* no. 7677) grew well, but no pycnospores were found. The tannin had an inhibiting influence.

E. radicalis mississippiensis grew well, but apparently was not able to use the tannin as food. No pycnospores were produced. The two cultures (nos. 2443 and 1196) used were very resistant to tannin, the former making the more vigorous growth.

From this series of cultures it appears that tannin (MERCK) affects various species of the genus *Endothia* quite differently. *E. parasitica* may for a time be retarded in its growth, but subsequently it feeds on the tannin, using the entire supply in the cultures tested. At the other extreme is *E. radicalis mississippiensis*, which appears to be entirely unaffected by tannin, and does not feed upon this substance. The cultures labeled *E. radicalis* (and CLINTON'S *E. gyrosa* no. 7677) are inhibited by the action of tannin. This is

true of the American strains to a greater extent than of the European.

GROWTH ON KERR'S EXTRACTS

Extract "1–X" is described by KERR as the water soluble tannin of the chestnut bark. It is insoluble in alcohol and in similar solvents. This occurs in quantities of 3–5 per cent in the bark. The sample used was between 95 and 100 per cent pure. The quantity available would not allow as extensive a series of cultures as were used for commercial tannin products. Accordingly, the percentages used were 1, 1.2, 1.6, 2, and 2.4 per cent. The agar remained firm in all cases. Inoculations were made with *E. parasitica* (STEVENS no. 1158), *E. radicalis*, and *E. gyrosa* (CLINTON'S no. 7674).

E. parasitica (American) gave an abundant aerial growth during the third week and of pycnospores during the fifth week. The great growth of pycnospores was on 2 per cent, which is about the normal amount in the bark. This extract is stimulating in normal and subnormal amounts.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) gave a growth similar to *E. parasitica*. At the end of the first week the most normal growth was on 1.2 per cent and the maximum on 2 per cent. Pycnospores appeared in 10 days and were present on all cultures at the end of the fifth week. Finally, it may be said that small quantities of this extract are stimulating, and that higher percentages produce vigorous growth of aerial mycelium and reduced number of pycnospores. It is rather surprising that this extract, which is so near pure tannin, is not as toxic as the commercial tannin.

Extract "2–X" is in all its reactions similar to that designated "1–X," except that it is soluble both in water and in alcohol. Its effect on agar is quite different, however, showing a tendency to digest proteids as do acids, and so render the medium viscous. A series of cultures was prepared containing 1, 1.2, 2, and 2.4 per cent of the extract, and inoculated with *E. parasitica* (STEVENS no. 1158) and *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674). The cultures of the two upper members of the series were quite noticeably viscous.

E. parasitica (American) gave growth on 1–1.6 per cent in one week, on 2 per cent in two weeks, and on 2.4 per cent in four weeks. Pycnospores were produced rather abundantly on 1.6–2.4 per cent at the end of two months.

Endothia radicalis (CLINTON'S *E. gyrosa* no. 7674) gave a good growth on 1.6 per cent in one week, and on 2 per cent three days later. In two weeks the growth was greater than with any other extract used, the maximum on 1 per cent and 1.2 per cent. Pycnidia began to appear on 1.2 per cent in about three weeks and finally on 1 per cent and 1.6 per cent, the maximum on the last percentage.

It is very evident that extract "2–X" is not so favorable for the growth of the fungus as extract "1–X." Both these results are surprising when compared with those obtained with commercial tannin.

Extract "3–X" is the coloring matter of the bark. While this is estimated as tannin in bark analysis, its real nature is unknown. It precipitates gelatin and combines with hide, but does not give the same distinct reactions with metallic salts as do other tannins. The sample used was between 85 and 90 per cent pure. As the quantity available was very small, it was used only in the proportion of 1 and 2 per cent. Both *E. radicalis* (*E. gyrosa* CLINTON no. 7674) and *E. parasitica* (STEVENS no. 1158) were grown on these media.

E. parasitica (American) gave a slight growth on 1 per cent of the extract in one week. This growth increased slowly, but finally became abundant. Germination was retarded for about 10 days on 2 per cent of the extract, and the growth was never so good as on 1 per cent. Pycnospores were found on cultures containing as much as 1 per cent of the extract during the first week, but were never found on the medium containing 2 per cent.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) gave a growth very similar to that of *E. parasitica*, but at the end of two months there were few pycnospores on the 1 per cent extract.

This extract was very toxic; the growth was always unhealthy and the production of pycnospores greatly checked. These results are surprising in that this extract, which is primarily coloring matter

of the bark, which under ordinary methods of analysis is estimated as "tannin," is more toxic than commercial tannin. KERR in commenting on these results, says:

> The action of "3–X" is also surprising, as it is what we term the coloring principle of the bark, the exact nature not having been determined by anyone that I know of. Its action brings out a rather interesting point, and that is that chestnut trees of northern growth, say on a line north of the southern boundary of Pennsylvania, contain very materially less coloring matter than the growth south of it, and, as we all know, the wood in the latitude referred to seems to have been more susceptible to the disease than that further south [letter December 26, 1913].

GROWTH ON COMBINATION OF KERR'S EXTRACTS

Since "1–X," which is a tannin extract, was stimulating, and "3–X," which is primarily coloring materials giving tannin reactions, was toxic, it was decided to combine the two into one extract. The material was made up into a series of cultures containing 0.2, 0.6, 0.8, 1.2, 1.6, 1.8, 2, 2.2, 2.4, 2.6, and 2.8 per cent. Sowings were made with *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674), *E. radicalis mississippiensis* (STEVENS no. 2424), and both American and Chinese strains of *E. parasitica*.

There was a tendency for the agar containing as much as 1.2 per cent of the extract to become less solid, but even with 2.8 per cent there was no approach to a real liquid condition.

E. parasitica (American) made a fair growth on 0.6 and 0.8 per cent in one week, with a slight growth throughout the series except on 0.2 and 2.2 per cent. The growth increased, but was relatively the same on the different cultures throughout the entire period. During the third week pycnidia appeared on 0.6 per cent, and by the end of the fifth week had developed on all cultures up to 2.2 per cent. In cultures containing more than 2.2 per cent the pycnospores decreased. The growth was always subnormal, but not so pronounced as in some other cultures.

E. parasitica (Chinese) also failed to grow on 0.2 and 2.3 per cent, but made some growth on 0.6, 0.8, and 1.2 per cent during the first week. At the end of the second week the cultures containing 0.6 per cent showed a subnormal growth, and the 2.8 per cent a very slight growth. The growth generally was less than that

of the American strain. Pycnospores were not found on either the check or the cultures containing tannin.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) made a fair growth on 0.2, 0.6, and 0.8 per cent, and a slight growth on the higher percentages. The growth generally increased on all cultures, but was always subnormal; no pycnospores were found.

E. radicalis mississippiensis grew on only the 0.8 per cent extract during the first week. In 10 days there was good growth on both 0.6 and 0.8 per cent, fair growth on 1 and 1.6 per cent and slight growth on 2 and 2.8 per cent. The growth increased on all cultures during two months' observation, and pycnospores were produced in abundance on all cultures up to 2 per cent.

In general it may be said that *E. parasitica* and *E. radicalis* thrive fairly well on cultures containing these extracts, but not so well as on the checks. The American strain of *E. parasitica* is more resistant that the Chinese strain, and *E. radicalis mississippiensis* is the most resistant of any species used.

KERR'S extract "A" is a compound of various forms of tannin and of other more or less related substances. It represents about 9 per cent of the dry weight of the bark. Its composition is as follows: tannin (containing the forms represented as "1–X," "2–X," and "3–X") 60 per cent, fermentable sugars 10 per cent, gallic acid 7 per cent, pentoses and pentosans 8 per cent, water 5 per cent, undetermined 10 per cent. This extract produces a rather advanced proteid digestion, causing the agar to become semi-fluid, and in the higher percentages used a considerable amount of fluid was present. The series included 1, 1.2, 1.6, 2, and 2.4 per cent of the extract. Sowings were made with *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674) and *E. parasitica* (STEVENS no. 1158).

E. parasitica (American) made a slight growth on all cultures during the first week. In 10 days the growth was good on all cultures up to 2 per cent. After that time the growth was slow. At the end of the first month pycnospores began to appear on cultures containing 1 per cent, but did not appear on others. This organism made its best growth on this medium.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) made a slight growth on cultures up to 1 per cent. The growth was poor throughout

the entire time. A very few pycnospores were produced on the 1 per cent, but not on the others.

This was the most toxic extract used in the entire series of experiments.

In order to test the possibility of preparing from commercial sources a compound similar in its effects to KERR'S extract "A," a tannin compound was made as follows: tannin (MERCK) 60 gm., dextrose 10 gm., gallic acid 7 gm., arabinose 8 gm., total 85 gm. This material was added to the agar in the same manner as was the tannin. The series of cultures prepared were at intervals of 0.2–3.0 per cent. The effect on the proteids was the same for this substance as for tannin. The higher percentages digested almost all proteids so that the medium was a clear liquid. Sowings were made with *E. radicalis mississippiensis* (STEVENS no. 2424), *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674), *E. parasitica* (American, CLINTON'S no. 7675, and the Chinese strain).

It seems scarcely necessary to give a detailed statement of these results. In all cases, except the American strain of *E. parasitica*, pycnidial formation was greatly retarded. Growth was very similar to that obtained with MERCK'S tannin.

Summary

1. Results obtained with commercial tannin are not always comparable to each other or to those obtained from specially prepared extracts, because of variations in chemical composition and the presence of tannin-like substances other than tannic acid. Commercial tannins of the same brand differ in their behavior in culture media, as indicated by the growth of the various species of *Endothia* used in these experiments.

2. Commercial tannin and tannin in the plant are not the same. No extract will be the same as the substances in the plant.

3. Tannin is an anhydrous glucoside of gallic acid and is easily converted by hydrolysis into gallic acid and related substances. It is very doubtful if any culture medium can be prepared containing as much pure, unchanged tannin as was put into it. Therefore, we cannot know the exact percentage of tannin in a culture medium,

but we can put known amounts into those with which we are working.

4. The quantity and form of tannin compounds present in the substratum each exerts an influence on the growth of the fungus; but when the fungus attacks a plant, we have no way of knowing the form of the tannin with which it comes in contact. However, it is quite evident that the tannin of the plant is associated with coloring materials and other substances, some of which are toxic. Furthermore, the fungus may be selective during either a part of or during its entire existence, and send its mycelium into certain tissues containing little or no tannin. *E. parasitica* is especially destructive because it works in the cambium cells, but later in life it pushes through the outer tannin-bearing cells of the bark for the production of its spores.

5. The character of the food supply influences the vigor of the fungus, and therefore its power to resist the toxicity of the tannin and other materials with which it comes in contact. The amount and character both of the food supply and of the tannin and other materials no doubt vary with the seasons and the growing periods of the host plant.

6. In almost every instance, without regard to the form of tannin used or the fungus grown, a high percentage (0.8 per cent or more) of tannin caused a retardation of germination, frequently followed by an abnormal stimulation to growth of aerial mycelium.

7. Species of *Endothia* show a marked response to the presence of tannin and related substances in the culture medium.

8. The species of *Endothia*, and to a certain extent strains of the same species, show a considerable variation in their response to tannin and other substances. (*a*) *E. radicalis mississippiensis* was unaffected by the tannin, but did not use it for food and did not produce pycnospores in cultures containing tannin. (*b*) *E. parasitica* was slightly retarded in its germination and early growth, but later was able to use as much as 2 per cent tannin as food. It was the only species studied that was able to utilize any considerable amount of tannin for food. *The American strains were more resistant to tannin and associated toxic materials than the Chinese strains.*

(*c*) *E. radicalis* (including *E. gyrosa*) was very susceptible to the influences of tannin.

9. Tannin (MERCK) affects the various species of *Endothia* very differently. *E. radicalis* (including *E. gyrosa*) is inhibited; *E. parasitica* is at first retarded and later is able to feed on the tannin; *E. radicalis mississippiensis* is practically unaffected and does not feed on tannin.

10. Analyses of chestnut bark made by KERR show a corresponding tannin reduction in diseased areas, which confirms the culture experiments and makes it possible to state that *E. parasitica* is able to use the tannic acid for food.

11. *E. parasitica* appeared to have its power of pycnidia production stimulated by commercial tannin and the true tannin extracts of chestnut bark, but reduced or inhibited by those extracts of chestnut bark which are composed almost entirely of coloring substances, but which are present in tannin extracts and estimated as tannin.

12. Specially prepared extracts of pure tannin were either stimulating or only slightly toxic when combined with coloring materials and other substances associated with tannins and responding in the same or in a similar manner to tannins. (*a*) KERR's "1–X" extract has a stimulating effect on *E. radicalis* (*E. gyrosa*) and *E. parasitica*. (*b*) KERR's "2–X" extract has a tendency to retard *E. radicalis* (*E. gyrosa*), *E. parasitica* (both American and Chinese strains), and *E. radicalis mississippiensis*. (*c*) KERR's "3–X" extract was extremely toxic to *E. radicalis* (*E. gyrosa*) and *E. parasitica*. (*d*) In *E. radicalis* (*E. gyrosa*) conidia production was at its maximum at 1–1.2 per cent of the extracts designated "1–X," "2–X," and "3–X," while but few if any pycnospores were produced on the other substances used. (*e*) The European strain of *E. radicalis* showed similar results, but the American strain showed a tendency to remain sterile. (*f*) A combination of "1–X" and "3–X" is somewhat toxic, but the toxicity of "3–X" appears to be largely overcome by the stimulating influence of "1–X." *E. radicalis* (*E. gyrosa*) and *E. parasitica* were slightly retarded, and *E. radicalis mississippiensis* was very slightly retarded. The Chinese strain of *E. parasitica* was less resistant than the American

strain. (*g*) KERR's "A" was most toxic of all compounds on *E. radicalis* (*E. gyrosa*) and *E. parasitica*. (*h*) Tannin compounds gave results similar to MERCK's tannin instead of "A."

13. A supernormal growth of aerial mycelium was usually accompanied by a corresponding reduction in pycnidia formation.

14. Harmful effects of tannin were also frequently shown by the absence of natural pigment from the mycelial pellicle and by the ashen color of the aerial hyphae. One or both of these might be present in the same series of cultures.

15. While tannic acid is no doubt toxic to many parasitic fungi, there are also other substances associated with tannin which are toxic. Some of these substances respond to the ordinary tannin tests and have probably been mistaken for tannin. The factors which enable plants to resist the attacks of parasitic organisms present an extremely complicated problem. The solution of this problem lies in the study of the chemistry and physiology of the cell.

16. Throughout the summary the terms "tannin" and "tannic acid" have been used in the generally accepted sense, but experiments with KERR's extracts "1–X," "2–X," and "3–X" indicate that the toxic property is in the coloring material, which in analytical work is usually estimated as tannin.

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RUTGERS COLLEGE AND
NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, N.J.

STUDIES OF DIOSPYROS KAKI. I

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 209

KONO YASUI

(WITH PLATES XII AND XIII AND ELEVEN FIGURES)

Although the Ebenaceae are recognized as a primitive family of the Sympetalae and possess several remarkable features, the only morphological investigation of them has been that of Miss HAGUE[1] on *Diospyros virginiana*. My purpose in undertaking the investigation of *D. Kaki* was to answer the following questions: (1) Are the Ebenaceae really primitive among the Sympetalae? (2) Are there cytological differences among the so-called garden varieties of *D. Kaki?* (3) How are the garden varieties related to one another? (4) How have they been obtained?

Material and method

D. Kaki is in very common cultivation in Japan and is represented by many so-called garden varieties. The fruits of these varieties differ in shape, size, and flavor, and also in their seeds. Each variety has also its characteristic flowers, leaves, etc., to which horticulturists paid little attention in classification, as compared with the differences in fruits and seeds. For example, the horticultural classification recognizes two principal divisions, dependent upon flavor of the fruit, as follows: (1) astringent kaki (shibu-kaki), and (2) sweet kaki (ama-gaki).

Each of these two divisions is divided into four or five groups, based upon the shape of the fruit, as follows. The astringent group contains (*a*) long, (*b*) round, (*c*) square, and (*d*) flat types; while the sweet group contains (*a*) gosho, (*b*) long, (*c*) round, (*d*) square, and (*e*) flat types.

In her report on *D. virginiana*, Miss HAGUE makes the following statement: "So far as the trees from which material was collected

[1] HAGUE, S. M., A morphological study of *Diospyros virginiana*. BOT. GAZ. 52:34–44. *pls.* 1–3. 1911.

were observed, they were dioecious, and bore only perfect flowers. One possible exception has been found recently." In *D. Kaki*, on the other hand, there are many such exceptions, as follows.

1. In some varieties only the pistillate flowers occur in good condition, while all the staminate and some of the pistillate flowers are imperfectly developed.

2. In other varieties (gosho, egosho, yamagaki, jenjimaru) both kinds of flowers are always well developed.

3. A tree in Mr. YABE'S garden in Tokyo (no. 1 of my material) has the same type of fruit as in some of the varieties in group 1, but has pistillate, staminate, and perfect flowers, and produces also two types of fruit from the two kinds of flowers.

4. No tree has been discovered by the writer on which only staminate flowers occur.

5. Some varieties have pistillate flowers only, when the trees are young, but as they become older or weaker (on account of poor nutrition or transplanting) they begin to produce staminate flowers also (hyakume-gaki).

6. Some trees show an alternation of fruiting and non-fruiting years, which means that during one year they produce many pistillate flowers and many fruits, while during another year they produce a few pistillate flowers and abundant staminate flowers. Under the latter conditions they bear very little fruit, or no fruit at all.

It may be added, in reference to the fourth condition referred to, that in Japan the staminate trees are being continually destroyed unconsciously, because the better varieties are produced always by grafting, and therefore many seedlings of staminate plants are destroyed, and pistillate plants take their place. The foregoing data gave me the suggestion that *D. Kaki* is naturally a monoecious plant (fig. 1), and that it is in process of losing this character, producing staminate flowers under cultivation.

I have selected five different varieties from one hundred or more for this study, as follows.

No. 1.—I am not certain of the name of this variety, but it resembles "yemon," which is an astringent, flat type. The difference is that yemon has no staminate flowers at the Okitsu

station, but this form has three kinds of flowers upon the same individual.

No. 2.—Tenryubo, which is a sweet, round type. It has no staminate flowers at the Okitsu station. The fruit is rather small and has many seeds.

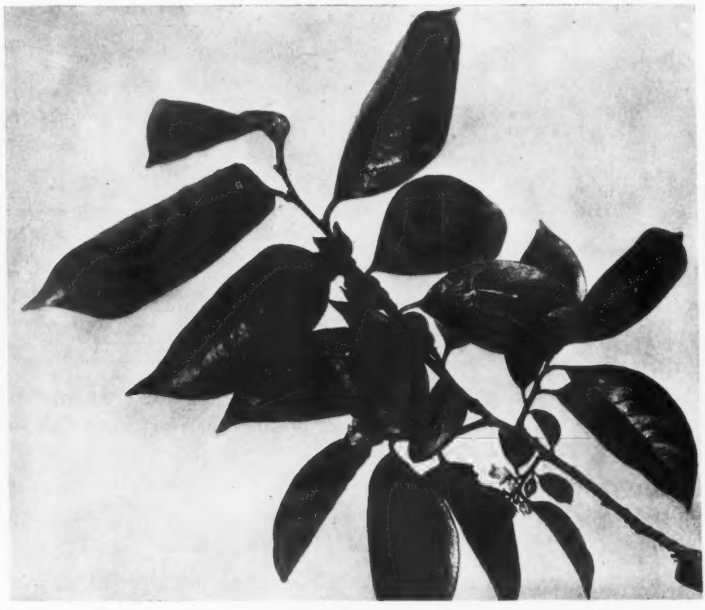


FIG. 1.—Branch of Tsuru-no-ko (one of the varieties of *D. Kaki*), showing the vigorous upper branchlets with pistillate flowers and the lower small branchlets with staminate flowers; $\times\frac{1}{2}$.

No. 3.—Jenjimaru is a sweet round type; but in this case there are always staminate flowers. The fruit is small and with many seeds, as in no. 2.

No. 4.—Tanenashi (meaning seedless) is an astringent square type. It has no staminate flowers and is generally seedless.

No. 5.—Fuyū is a sweet, gosho type. Usually it has no staminate flowers, but occasionally a very few may be produced. Occasionally the anthers of the perfect flowers contain a number of

pollen grains, which germinate well on the stigma and in nutrient fluids.

I have also examined many other specimens, but the main results of the investigation were obtained from these five. The material of no. 1 was collected in Tokyo, but the others were obtained from the experiment station at Okitsu, in Central Japan. The material was fixed with Flemming's weaker solution, and chromo-acetic acid mixture was also used, giving better results than the former medium. The sections were stained with Flemming's triple stain and Haidenhain's iron-hematoxylin.

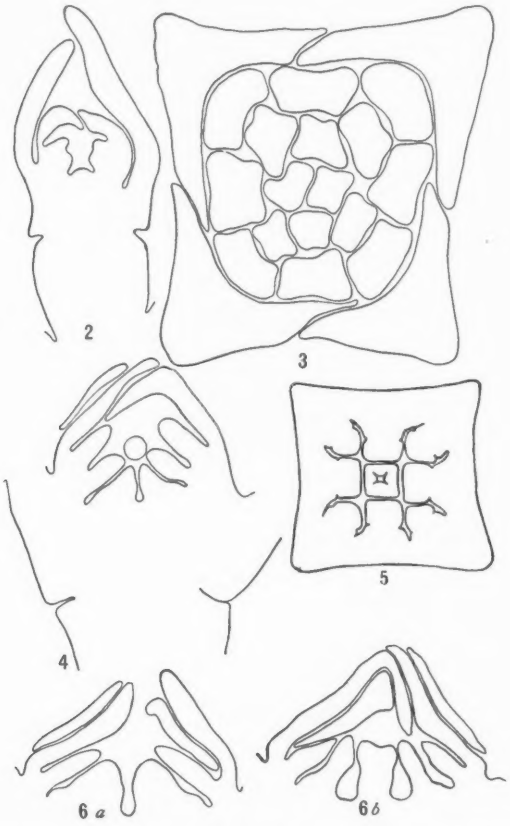
Development of staminate flower

The cluster of staminate flowers appears on young shoots in early spring. It consists usually of three flowers (fig. 1), but sometimes includes 7 or more. The very young shoot bends downward, and 2–4 of the lower leaves have nectaries on the under surface, near the base. The succession of members is strictly centripetal. The calyx is valvate, but the four petals are spirally arranged (fig. 3). The 16 stamens occur in four cycles (fig. 3), and appear in pairs from the earliest stage (fig. 2). The mature stamen has a very short filament, and the connective extends above the anther. The pistils complete their development very early. A few flowers were found which were 5-merous instead of 4-merous.

Development of perfect flower

The perfect flower always occurs at the top of the cluster. It has the same arrangement of members as the staminate flower when young (fig. 4), and the pistils of the perfect flower are smaller than those of the pistillate flowers. Occasionally they have less than 8 ovules, which is the ordinary number in the pistillate flowers, and sometimes they have no ovules. Abnormalities occur, not only in the number of ovules, but sometimes in the irregular forms of the pistils themselves. The fruit of the perfect flower is always smaller than that of the pistillate flower, and usually it has no seeds.

The foregoing facts suggested that the perfect flower is an abnormal one, produced by favorable conditions of nutrition; and that therefore it does not indicate a general tendency.



FIGS. 2–6.—*D. Kaki:* fig. 2, longitudinal section of a young staminate flower of no. 1; fig. 3, transverse section of a young staminate flower of no. 1, with calyx removed; fig. 4, longitudinal section of a perfect flower of no. 1, with calyx removed; fig. 5, transverse section of the lower part of a pistillate flower of no. 4, showing the 8 anthers and a pistil; fig. 6, flower of no. 4: *a*, radial section of a young pistillate flower through the two large opposite protrusions; *b*, longitudinal section through the two initial papillae on the large protrusion.

Microspore formation

There is no marked difference between the primary sporogenous cells and the primary wall cells before the latter begin to divide. Two periclinal divisions give rise to three outer wall layers and a tapetal layer. Two or three successive divisions separate the primary sporogenous cells from the mother cells. At the same time the uninucleate tapetal cells become very distinct between the sporogenous tissue and the wall layers, the latter of which become more and more flattened.

During the comparatively long resting period, the spore mother cell increases in size, its protoplasm becomes denser (but not so dense as that of the tapetal cells), and the nucleus becomes large (pl. fig. 1). The nucleus contains rather a small amount of chromatin granules associated with the fine linin network, and also a remarkably large nucleolus and several small ones (pl. figs. 2, 3).

In some cases I observed the larger chromatin granules appearing in pairs, but this did not seem to be the usual situation. The reticulum is denser at the outer part of the nuclear cavity than within. During presynapsis certain connections between the chromatin granules disappear, so that the reticulum gradually becomes simpler in structure. At the maximum of synapsis the chromatin substance appears like a mass of granules (pl. figs. 4 and 5).

There is no morphological connection between the chromatin reticulum and the nucleolus, although they seem to occur in very intimate relationship. A great many nucleolus-like bodies were observed close to the chromatin thread, near the large nucleus, but in other cases there was no such relationship (pl. fig. 4). In the presynapsis stage the double nature of the chromatin thread is not clear, but it becomes gradually evident with the loosening of the thread (pl. figs. 6, 7).

Before 1895, when MOORE called attention to the synapsis stage as an important period in the history of the nucleus, this stage was ignored. Since that time it has attracted chief attention, and is regarded in general as an important event in the history

of the nucleus. Some investigators, however, do not regard it in this light. For example, LAWSON[2] makes the following statement.

> During their development, however, there is a great accumulation of sap within the nuclear cavity, which causes a great osmotic pressure. The pressure, acting from within, causes the nuclear cavity to expand. This expansion continues until the nuclear cavity grows to twice or even three times the original size. As the growth proceeds the membrane is gradually withdrawn from the chromatin mass within. The result of this withdrawal of the nuclear membrane is the formation of a large clear area of nuclear sap containing the mass of chromatin which has been left at one side. No evidence whatever was found to show that any contraction of the chromatin had taken place.

In my material of *D. Kaki* I could not recognize such a remarkable enlargement after the chromatin reticulum began to separate from the nuclear membrane. The great enlargement of the nucleus occurs in the resting stage, so that there is no conspicuous difference in the size of the nucleus at the synapsis stage and at the end of the resting stage, as LAWSON indicates.

The synapsis stage is a normal occurrence, and during it there is some rearrangement, but not fusion of chromatin bodies, which results in the spireme stage. At the beginning of the spireme stage the thread is distributed in the outer cavity of the nucleus, where thickening and shortening occur, resulting in the diakinesis stage (pl. figs. 6, 7, 8). There is no difference in the size of the pairing chromosomes, which are usually parallel, but sometimes X or V-shaped. At the same time there appear in the cytoplasm numerous fibers which penetrate the nuclear cavity and become attached to the chromosomes by one end, in connection with which the nuclear membrane begins to disappear. By the elongation of the fibers the chromosomes, which have been distributed on the surface of the nucleus e drawn into the center of the nuclear cavity, and there are arranged in an equatorial plate by the gathering of the free ends of the fibers at the poles (pl. figs. 9, 10, 11). The nucleolus disappears entirely during metaphase.

The number of chromosomes is difficult to count by reason of their small size. Usually there are 28 in pairs, but sometimes

[2] LAWSON, A. A., The phase of the nucleus known as synapsis. Trans. Roy. Soc. Edinburgh **47**:591–604. *pls. 2.* 1911.

27 in no. 1 (pl. fig. 11). In *D. virginiana* Miss HAGUE counted 30 or more.

The longitudinal division of the chromosomes in preparation for the homotypic division occurs in metaphase, and at telophase the chromosomes become arranged in the spireme and spread over the outer cavity of the daughter nucleus. The number of chromatin granules is not the same as the *x* number of chromosomes; and there appear two or three nucleoli in the daughter nucleus (pl. fig. 12, *a*, *b*). The homotypic division of the microspore mother cell occurs in the usual way. The two axes of the spindle fibers of this division are not in any definite relation to one another (pl. figs. 13, 14). In wall formation the microspores are in tetrahedral arrangement (pl. figs. 15, 16), and the mother cell wall, which becomes mucilaginous and separates the four young microspores (pl. fig. 17), disappears gradually with the formation of the thick wall of the pollen grain.

At the same time the uninucleate tapetal cells enlarge and become multinucleate. These divisions of the tapetal nuclei are mitotic at first, but later they become amitotic, after which they begin to disorganize when the homotypic division of the mother cell has been completed (pl. figs. 20, 21, 22).

The mature pollen grain is spherical, with remarkable canals upon the surface of the wall (pl. fig. 18). The cytoplasm occurs as a thin layer surrounding a large central vacuole. In general only a single nucleus was observed in the pollen grain, pl. fig. 19 representing the only specimen in which two nuclei were observed.

The foregoing account of microspore formation was obtained from material of no. 1. I have investigated no. 3 also, but could not determine the number of chromosomes. The pollen grain is a little larger than in the case of no. 1. The anther of no. 3 contained many withered pollen grains.

Development of pistillate flower

The pistillate inflorescence usually appears upon an upper branch, consisting of one flower and two bracts that suggest the position of flowers that have disappeared. The bracts usually

fall after blooming, but in some varieties they persist even after the ripening of the fruit.

The staminate flower begins to develop as early as the pistillate, but the pistils develop more rapidly than the stamens, resulting in the sterilization of stamens and in some cases in their disappearance.

The mature pistillate flower generally has two sets of stamens (nos. 1 and 2), but in some cases has more (nos. 1, 2, and some of 4) or less (no. 1 and some of no. 2).

I did not discover the following condition described by Miss HAGUE: "In the pistillate flower it is a common occurrence to find the number increased by the branching of one or more of the stamens."

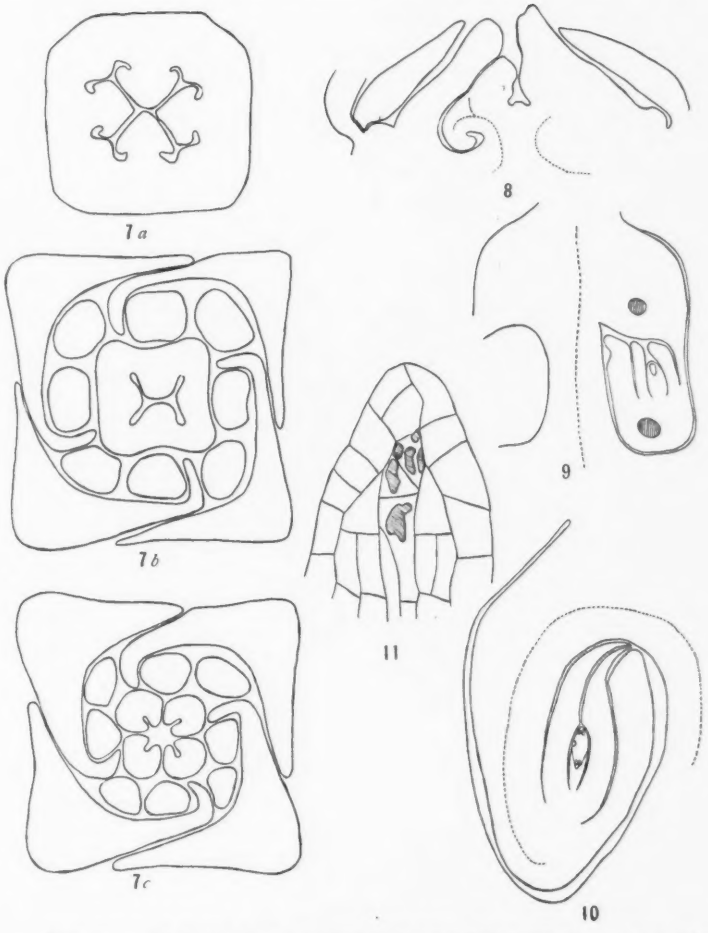
In the development of the pistil there appear first four large protrusions, and then four smaller ones between them (figs. 5 and 6, *a*, *b*). The larger ones give rise to two small papillae, which are the initials of ovules (figs. 6, *d*, and 7). The papillae grow at first toward the wall of the ovary, then curve downward toward the center, and finally upward (figs. 8 and 9). Simultaneously the small protrusions develop toward the center of the ovary, resulting, along with the development of larger protrusions, in 8 loculi for the 8 ovules.

The ovule has two integuments (fig. 9), which extend beyond the nucellus like a beak at the time the embryo sac is fully developed (fig. 10).

Megaspore formation

In all the varieties examined the archesporial cell is solitary and hypodermal. There is no division resulting in a parietal cell, so that the archesporial cell is the megaspore mother cell. The heterotypic division of this nucleus occurs in the same way as in the case of the nucleus of the microspore mother cell. After nuclear division, the cytoplasm organizes into two parts, the inner one being the larger. Sometimes a wall between these two parts does not appear before another division of nuclei occurs.

Wall formation in connection with the megaspores is variable in direction. Sometimes the two walls are perpendicular to one



FIGS. 7–11.—*D. Kaki:* fig. 7, cross-section of a pistillate flower of no. 4, at a little older stage than in figs. 5 and 6: *a*, lower part, showing 4 large protrusions which have papillae, and small protrusions among them; *b*, portion of the upper part, showing 4 petals, 8 anthers, and upper part of ovary; *c*, a still higher part, showing 4 petals, 8 anthers, and 4 young stigmas; fig. 8, longitudinal section of a young pistil of no. 4; fig. 9, longitudinal section of the central part of an ovary of no. 4, showing the longitudinal section of an ovule; fig. 10, longitudinal section of an ovule of no. 4; fig. 11, longitudinal section of the upper part of a nucellus of no. 4, showing the disorganizing embryo sac mother cell.

another, but at other times more or less parallel, resulting in different arrangements of megaspores (pl. figs. 23–26). The micropylar megaspores degenerate in connection with the maturation of the large innermost one in forming the embryo sac (pl. fig. 27). In my material I observed all of the megaspores disorganizing (fig. 11), and it is evident that this disorganization of the megaspore which ordinarily gives rise to the embryo sac is one reason for seedless fruit. In such a country as Japan, where many different kinds of persimmons are cultivated together, the difficulty of pollination is out of the question.

Summary

1. *Diospyros Kaki* is not a dioecious plant, but a monoecious one whose staminate flowers are disappearing under cultivation.

2. The monoecious habit might have been derived from a condition of perfect flowers; therefore this habit is not a primitive character in this species.

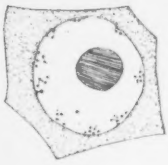
3. Perfect flowers do not indicate the primitive character of the variety in which they occur; they appear among other varieties only through restoration of lost parts.

4. The primitive character of Ebenaceae among Sympetalae is indicated by the spiral arrangement of petals; the stamen situation, although the number of stamens is not definite; and the two integuments.

5. Megaspore formation is also of a primitive character, and suggests, along with other characters of the family, that it may have some relation to the Myrtiflorae.

6. There is no parietal tissue in the microsporangium, which indicates that Ebenaceae come from some higher family of Archichlamydeae, because in the lower families parietal tissue usually occurs in the megasporangium.

7. Embryo sac formation occurs in the usual way, and in general furnishes no evidence for the evolution of dicotyledons. It is true in general, of course, that the gametophytes of angiosperms are of less value for evidence concerning evolution than the sporophytes.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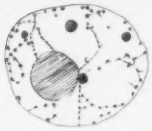
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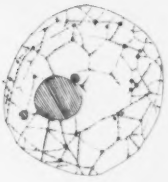
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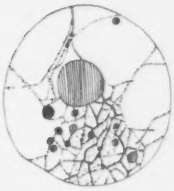
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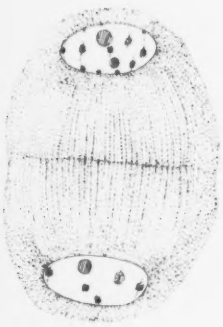
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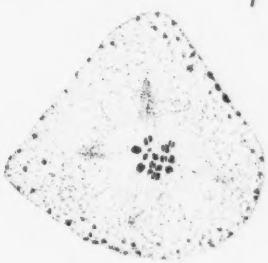
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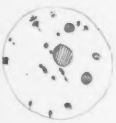
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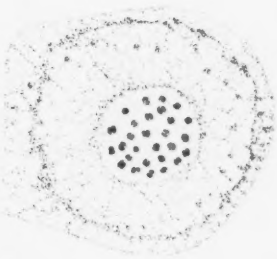
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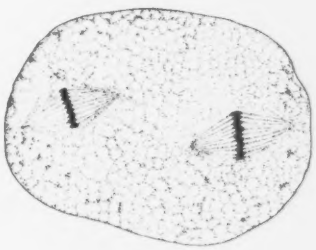
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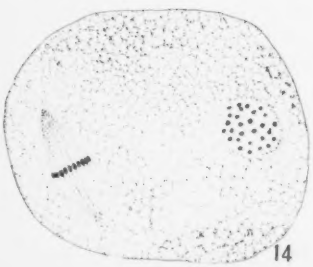
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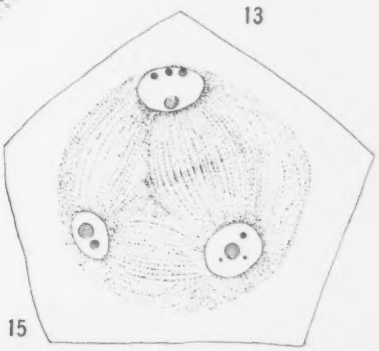
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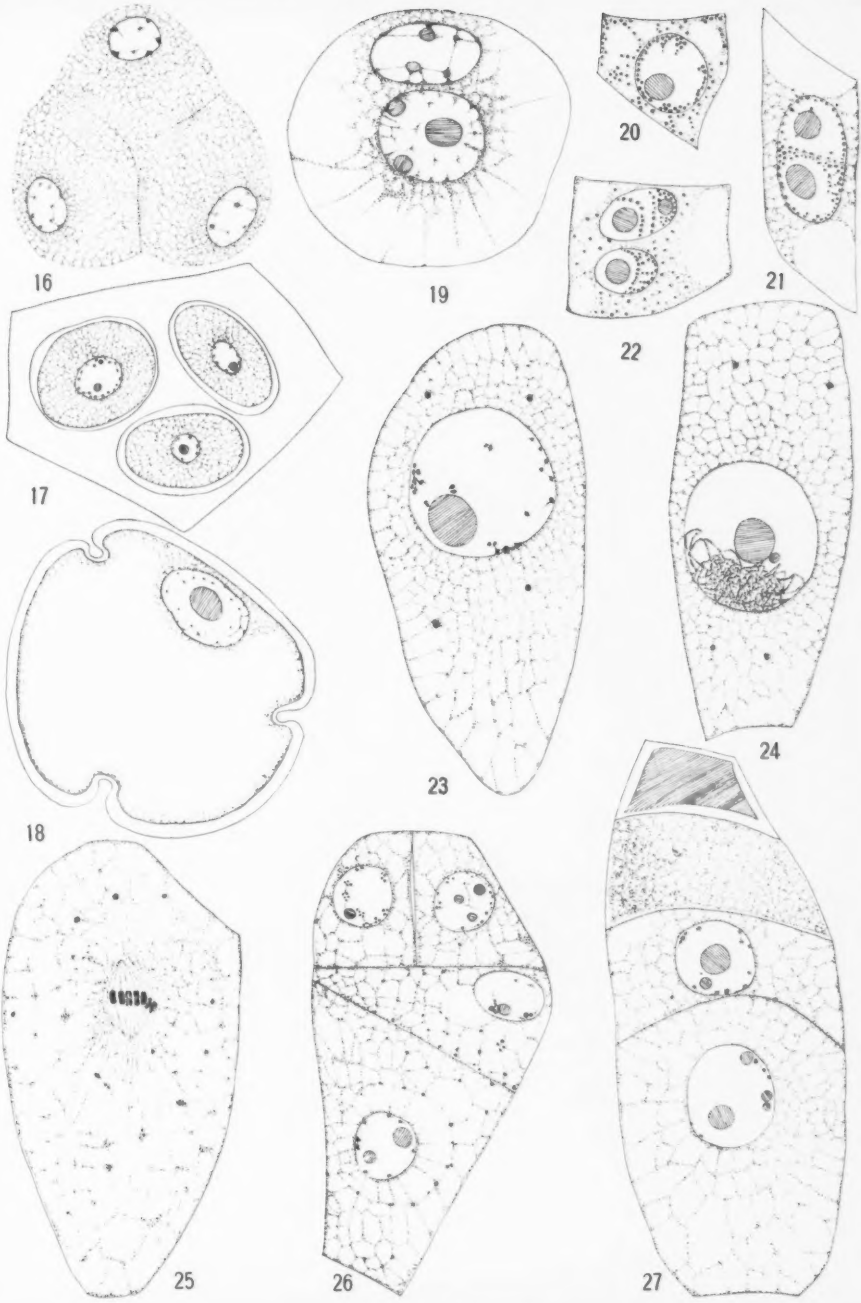
YASUI on DIOSPYROS



YASUI on DIOSPYROS

8. The $2x$ number of chromosomes is 56 or 54.

I desire to express my thanks to Professor JOHN M. COULTER, under whose direction the investigation was undertaken; to Professor C. J. CHAMBERLAIN, who gave much advice; and to Mr. Y. KUMAGAI and Professor Y. YABE, who assisted in securing material.

UNIVERSITY OF CHICAGO

NEW AND NOTEWORTHY PLANTS FROM SOUTHWESTERN COLORADO

EDWIN PAYSON

During the summer of 1913 and the spring and summer of 1914 it was the writer's privilege to make a collection of the plants of western Montrose County, from the Uncompahgre Plateau on the east to the La Sal Mountains of eastern Utah on the west. Of all parts of the state, the southwestern portion is the least known botanically and may therefore be expected to furnish novelties to the systematist for many years to come. This is especially true of the spring flora of the arid or so-called "Upper Sonoran" life-zone. The reason for this is obvious when one considers that most of the collections have been made upon the summer flora, and that by the middle of June most of the vegetation of the arid district has withered and even the seeds have scattered. Though the collections of 1913 and 1914 were made upon the montane as well as the desert flora, most of the interesting forms were discovered at the lower altitudes. The undescribed species, those that had not previously been reported from the state or had been omitted from recent manuals of the district, as well as some perplexing forms, are treated in this paper. A complete set of these plants, containing the types of the species and varieties characterized, is deposited in the Rocky Mountain Herbarium.

CALOCHORTUS FLEXUOSUS Wats.—This lovely and interesting mariposa lily, which has heretofore been known only from farther south and west and has not generally been credited to Colorado, was collected in bloom near Naturita on May 12 and May 26, on a red clay hillside over which it was growing in great profusion. The stems were stiff and flexuous and showed no inclination to become prostrate or twining. The flowers have been described as "purple" or "purplish," but as a matter of fact they are at first white and later become a very delicate pink; it is only in withering that they become "purplish." Nos. 289 and 357.

SALIX.—Nos. 288 and 289, collected in flower near Naturita on May 9, 1915. The stamineal filaments are united nearly to the summit in these specimens. If it were not for this character, I would refer this willow to *S. cordata* Muhl., but because of knowledge of material from one plant only and the lack of mature leaves, I prefer to leave it undetermined for the present.

ERIOGONUM MICROTHECUM Nutt.—Collected near Naturita, September 26, 1914, on a dry rocky hillside; no. 613.

POLYGONUM AMPHIBIUM L.—A form of this species with long fringed sheaths is common in ponds on the Uncompahgre Divide in Tabeguache Basin; no. 173.

Aquilegia pallens, n. sp.—Perennial: stems slender, erect, glandular, pubescent, and sparsely villous throughout; radical and lower cauline leaves biternate; petioles slender, only somewhat glandular, moderately villous, much exceeding the rest of the leaf; leaflets rather thick, broadly cuneate to suborbicular, base often quite truncate, margins inclined to be revolute, under surface slightly glaucous, more viscid and hairy than upper one; upper cauline leaves once or twice ternate, only the few small floral bracts entire: flowers about 3 cm. in diameter and 5 cm. long, mostly erect; sepals 15 mm. long, white or pale blue, lanceolate with narrow claw; petals white, blades about 8 mm. long and 6 mm. broad, truncate, spurs straight, about 3 cm. long; stamens exceeding the petals and exceeded by the mature styles; ovaries pubescent: mature fruit unknown.

Collected only in the canyon of La Sal Creek, Utah, within a few miles of the Colorado boundary, at an elevation between 6500 and 7000 ft. The plants were growing in a moist area at the foot of the sandstone walls of the canyon. The slender stems, the thick leaves, the viscid and villous herbage to which grains of sand were clinging, and the small white or pale blue flowers gave this plant of the Upper Sonoran life zone quite a different aspect from *A. coerulea* of alpine and subalpine stations to which it is evidently related. No. 443, June 16, 1914.

Cleomella montrosae, n. sp.—Glabrous annual, 8–15 cm. high, from a slender taproot: stem diffusely branched from base: leaves trifoliate; petiole 2–3 mm. long; leaflets linear-oblong, 10–14 mm. long, thickish, distinctly petiolulate, more or less folded and with

prominent midrib; stipules present, of two or three filiform setae: flowers small, yellow, crowded in corymbiform racemes terminating the branchlets; pedicels 2–3 mm. long, subtended by simple, linear, foliar bracts; calyx segments greenish yellow, 1 mm. long, lanceolate, weakly bristle-tipped; petals elliptical, thickish, three times as long as sepals; stamens exserted; ovary slightly obovate, with two ovules in each cell: capsule ovate, 3 mm. long, 2–3 times as long as the slender stipe and 4–5 times as long as the persistent style; seeds 1 or 2, dark brown, broadly obovate, 2 mm. long.

Probably most nearly related to *C. oocarpa* Gray, from which it is easily distinguished by the possession of a very short stipe. Collected on dry, loose, gypsiferous hillsides near Montrose, alt. 5800 ft., in fruit, October 1912. Flowers produced in May and June. No. 222.

Lupinus crassus, n. sp.—Perennial, caudex branched, forming a dense mat from which arise many stems: stems rather stout, erect, unbranched, sparsely sericeous, 3–4 dm. high: leaves five or six on a stem, green; petioles silky-pubescent, those of lower leaves 8–10 cm. long, of upper ones generally longer than the leaflets; leaflets 5–10 (usually 7 or 8), thickish, somewhat folded, cuneately obovate to oblanceolate, obtuse, silky-pubescent below, upper surface glabrate, 3–4 cm. long, 10–12 mm. wide: inflorescence erect, many-flowered; pedicels 4–5 mm. long, pubescent with short, spreading, white hairs; bracts purplish, lanceolate, 10–12 mm. long, exceeding the buds at the apex of the inflorescence and falling by the time the flowers are fully expanded: calyx not spurred, densely pubescent, lower lip entire, 7–8 mm. long, slightly exceeding the bifid upper lip; corolla white, fading brownish, 12 mm. long, distance from apices of banner and wings about 6 mm., keel included, triangular, acute, scarcely falcate, ciliate except at apex; ovary pubescent, ovules 7 or 8.

This splendid lupine was collected near Naturita, April 21, 1914. At this time it was not in full bloom. Its habit of growth is peculiar in that it forms a dense mat rather than a clump. It seems to prefer soils that are somewhat gypsiferous in character and is often found growing on a loose hillside. No. 239.

Lupinus fulvomaculatus, n. sp.—Perennial: stems nearly glabrous, several to many from a single root, branching upward and

forming a rounded clump 5–7 dm. high: leaves numerous, petioles of stem leaves about equaling the longest leaflets; leaflets 6–8, rather unequal, oblanceolate, not folded, green and glabrate above, sparsely pubescent and somewhat glaucous beneath, 4–6 cm. long, 8–10 mm. broad, obtuse: racemes numerous, terminating the branchlets, 2–3 dm. long; bracts deciduous; pedicels about 2 mm. long, softly pubescent as is also the calyx: flowers very small (7–8 mm. long and about 6 mm. deep), crowded and rarely somewhat verticillate, light blue with a conspicuous rich brown spot on banner; calyx neither spurred nor gibbous, strongly bilabiate, with two linear-lanceolate bractlets attached near sinus; upper lip 3 mm. long, 2-cleft, lower lip 4 mm. long, entire; banner nearly orbicular when spread out, 7 mm. in diameter, shorter than wings, distance between apices of banner and wings about 3 mm.; wings broadly oblong, exceeding the keel by 2 or 3 mm.; keel strongly curved, margins ciliate from middle to near the comparatively blunt, blue-purple apex: pods densely villous, 2–3 cm. long and 8–9 mm. wide; seeds 4 or 5.

A species with distinctive characteristics and peculiar aspect due to the numerous long racemes of very small, "compact," light blue flowers with the conspicuous brown areas on the banners. Collected only at Tabeguache Basin on the Uncompahgre Divide, alt. 8000 ft., where it is rather plentiful in open aspen groves or on brushy hillsides. Type no. 547, July 29, 1914.

ONOBRYCHIS SATIVA L.—Escaped from cultivation on upper La Sal Creek, Utah; no. 437, June 16, 1914.

ASTRAGULUS COLTONI Jones.—Common in Long Park, near Naturata among the junipers and piñons; no. 336, May 22, 1914.

Astragalus naturitensis, n. sp.—Perennial: gray with appressed pubescence throughout: stipules large, scarious, ovate to broadly lanceolate, free; leaves 5–7 cm. long; petioles 2–3 cm. long; leaflets 9–11, narrowly elliptical, more densely pubescent on lower side, 6–7 mm. long: inflorescence racemose-capitate in flower, elongating somewhat in fruit; peduncles equaling or exceeding the leaves, spreading: flowers few (6–10); calyx tubular, about 7 mm. long, appressed pubescent and sparsely nigrescent, teeth short (1–2 mm.), triangular-subulate; corolla 12–15 mm. long, conspicuously bicolored, standard white, 2 mm. longer than keel,

apical portion of lateral petals and blunt keel red: pods sessile, mottled with red or purplish blotches, horizontal or ascending, 2 cm. long, 6 mm. wide, acute, straight, linear-elliptic, flattened dorsally and on account of the intrusion of the dorsal suture very broadly cordate in cross-section.

This species is in general aspect so remarkably close to *A. desperatus* Jones that at first glance one would suppose them to be identical. Closer examination of the floral and fruit characters, however, show them to be quite distinct. In *A. desperatus* the standard, as well as the wings and keel, is red, while in the new species the flowers are conspicuously bicolored. JONES has described the pod of *A. desperatus* as being "long hairy"; the pods of the new species are short pubescent. No mention is made of any markings on the pod of *A. desperatus;* the pods of the new species are very conspicuously mottled.

Collected on dry, rocky mesas near Naturita; alt. 5400 ft.; May 27, 1914; no. 360.

Astragalus amplexus, n. sp.—Biennial or short-lived perennial: stems 3–4 dm. high, several from vertical root, sparsely pubescent, erect or spreading, more or less flexuous and often serpentine: leaves comparatively few, 8–12 cm. long; leaflets 17–21, oblanceolate, truncate or retuse at apex, those of lower leaves opposite, of upper often alternate, 9–12 mm. long, 4–6 mm. broad, green, glabrate above, sparsely pubescent below; stipules 3–4 mm. long, united only at base: racemes axillary to and not surpassing the leaves, 7–10 cm. long: flowers 12–15, horizontal, pale violet when fresh; calyx tube cylindric-campanulate, 6 mm. long, nigrescent; lobes linear, 2–3 mm. long; banner scarcely reflexed and inclosing the keel and wings; wings about 14 mm. long with slender claw half as long as the narrow blade; keel about 2 mm. shorter than wings, blade about 2 mm. wide, very blunt: pods sessile, sparsely pubescent, completely 2-celled, coriaceous, 2–2.5 cm. long and 6 mm. in diameter, neither suture intruded and hence pod nearly circular in cross-section, arcuate dorsally, mottled on dorsal surface with irregular purplish splotches.

This species evidently belongs to the MICRANTHI section of WATSON'S revision of the genus in KING'S *Report,* and to the genus *Astragalus* as restricted by RYDBERG in his segregation of the genus in Bull. Torr. Bot. Club **32**:657. 1905. Collected on dry hillsides in Long Park near Naturita, May 22, 1914; alt. about 6000 ft. (apparently infrequent); no. 335.

Psoralea aromatica n. sp.—Perennial herb, 1.5–2 dm. high: stems decumbent and sparingly branched, 2–3 dm. long, sparsely pubescent with short, rigid, appressed hairs: leaves green, rather numerous, on petioles 3–5 cm. long; leaflets usually five, petiolulate, cuneate-obovate, obtuse, mucronate, 2 cm. long, 1 cm. broad, lepidote, sparingly appressed pubescent; stipules lanceolate and scarious, persistent, 6–8 mm. long: flowers borne in axillary racemes 1.5–2 cm. long, light blue, in part at least; bracts linear-lanceolate, scarious, much shorter than the calyx; calyx somewhat accrescent in fruit, bilabiate, sparingly pubescent except on the margins where it is densely pubescent with short rigid hairs, lower lip lanceolate, nearly equaling the corolla, upper lip 2-cleft, almost one-half as long as the lower division, lateral lobes 2 mm. long; corolla 1 cm. long, blade of standard broadly elliptical; lateral petals narrow, scarcely equaling the standard; keel much shorter (7 mm. long): pods 1.5 cm. long, bearing a broad, slightly curved acute beak 8 mm. long; seed smooth, compressed, elliptical, 4–5 mm. long.

This plant is perhaps most closely related to *P. californica* Wats., from which it is easily distinguished by the glabrate calyx with its very unequal lobes. The name is given because of the delicate but pleasant odor of the dried plants. Collected on a dry, clay hillside near Bedrock, Paradox Valley, Colorado; alt. about 5000 ft.; no. 451, June 17, 1914.

EUPHORBIA FENDLERI **dissimilis,** n. var.—A well marked variety, differing from typical *E. Fendleri* T. & G. principally in the larger ovate to lanceolate leaves (7–10 mm. long) which are often acute, and in the usually entire appendages which are 1–3 times as broad as the gland.

Common on dry rocky hillsides, throughout the western half of Montrose County; no. 119, June 27, 1913, and no. 493, July 13, 1914, at Naturita, Colorado.

RHUS UTAHENSIS L. N. Gooding.—Collected on a rocky hillside near Bedrock, Paradox Valley; alt. about 5200 ft.; June 17, 1914; no. 457.

OROGENIA LINEARIFOLIA **lata,** n. var.—Leaves elliptic and quite entire or irregularly lobed and cleft at apex and then truncate, 4–6 cm. long, 1–3 cm. broad.

Growing with the species on dry flats in Tabeguache Basin; alt. 8000 ft. Intermediate forms were not seen. No. 369, June 1, 1914.

AULOSPERMUM BETHELI Osterhout.—Collected at Naturita on dry hillsides, April 27, 1914, where it is rather common. Previously known only from type locality near De Beque, Mesa County, Colorado. No. 225.

NAVARRETTIA BREWERI (Gray) Greene.—Typical plants of this species were collected in Tabeguache Basin, June 23, 1914; no. 459.

OREOCARYA TENUIS Eastwood.—Collected in flower in Long Park, near Naturita, on May 22, 1914; alt. about 6500 ft.; no. 337.

Oreocarya gypsophila, n. sp.—Densely cespitose perennial: caudex much branched, woody, and clothed with the petioles of former leaves: radical leaves crowded, narrowly spatulate, usually folded, 2–3 cm. long, obtuse, canescent with short, stiff, appressed hairs which on the dorsal surface are often pustulate at base; blade 3–4 mm. broad; petioles ciliate with coarse white bristles; stem leaves reduced upward, uppermost broadly linear, passing into inconspicuous, lanceolate bracts: stems slender, 6–10 cm. high, moderately hirsute: inflorescence short-hirsute, not becoming fulvous, thrysoid, the relatively few flowers somewhat capitate; pedicels short (1–2 mm. long): calyx consisting of five narrowly lanceolate, bristly divisions, 6 mm. long; corolla white, salverform, tube twice as long as the calyx lobes, limb 12 mm. in diameter, consisting of five subelliptical lobes, the divisions of which do not reach to the throat; crests present but not conspicuous; anthers less than 2 mm. long, filaments very short, attached about midway on the corolla tube: nutlets broadly ovoid, white, sharply rugose transversely, all of the nutlets developing.

This species is probably most closely related to *O. cristata* Eastwood. Collected on a dry gypsum hill in Paradox Valley, Colorado; alt. slightly over 5000 ft.; no. 458, June 18, 1914.

Pentstemon cyanocaulis, n. sp.—Glabrous and more or less glaucous perennial: stems usually one, 3–6 dm. high, erect and branched, often becoming blue or purple in the inflorescence: basal leaves spatulate, 4–7 cm. long; cauline leaves oblong-spatulate, sessile, passing gradually into the much reduced lanceolate

bracts: flowers in a somewhat secund, interrupted thrysus, 1–2 dm. long; sepals lanceolate, acute or acuminate, 4–5 cm. long, with a rather broad membranous margin; corolla usually horizontal, blue, scarcely ventricose-gibbous, obscurely bilabiate, 17–20 mm. long, lobes subequal; sterile filament sparsely hirsute for over half its length, slightly dilated; anthers bearing short, stiff hairs, cells not confluent, dehiscent from base to apex.

The closest relative of this species is apparently *P. strictus* Benth., from which it is easily distinguished by the shorter, broader leaves and the unusually small flowers for this group. Collected on high, dry mesas near Naturita, May 25, 1914, where it is rather infrequent; alt. about 5800 ft.; no. 348.

PEDICULARIS CENTRANTHERA A. Gray.—Rather common among junipers and piñons in western Montrose County at altitudes from 5800 to 7000 ft. Collected near Naturita, May 25, 1914; no. 345.

CHRYSOTHAMNUS FORMOSUS Greene.—This almost unknown species has apparently been collected but once before, by GREENE near Grand Junction, Colorado, in August 1899, and has been omitted from recent works on the flora of this region. When GREENE described it he did not know the floral characters and therefore I append a brief description of the species.

Low, branched from a woody base, forming dense mats 3–4 dm. high, branches permanently white tomentose: leaves narrowly filiform, somewhat revolute, tomentose but becoming greenish with age, 3–4.5 cm. long: inflorescence cymose, heads rather large and showy; involucres nearly or quite glabrous, their bracts imbricated in distinct vertical rows, 4–5 bracts in each row, ovate to oblong-linear, subacute: corollas usually five in a head, 1 cm. long, the pubescent tube gradually enlarging to form the somewhat inflated throat; tube and throat subequal, lobes short, less than 1 mm. long, scarcely acute; anthers equaling the corolla; style branches exserted, appendages subulate-filiform, three times as long as the stigmatic portion: pappus somewhat deciduous, dull white; achenes pubescent.

Collected near Naturita, alt. 5400 ft., on red clay hillsides that were more or less alkaline in character; September 8, 1914; no. 605.

Helianthella scabra, n. sp.—Stems several, 6–8 dm. high, from a woody, perennial root, somewhat cymosely branched above and bearing many rather small heads: leaves dark green, coriaceous, prominently nerved, linear-lanceolate at base to nearly linear in the inflorescence, hispidulous-scabrous (occasionally glabrate above), acute or obtuse; radical leaves 15–25 cm. long, attenuate into a petiole two-fifths the length of the blade; cauline 5–10 cm. long, short-petioled to sessile, first pair opposite: peduncles whitened with minute pubescence; involucral bracts more or less pubescent, in about three series, mostly lanceolate and shortly acute; ray flowers 8–10, neutral ray flowers bearing orange-yellow ligules over 1 cm. long, oblong, entire or cleft at apex, somewhat recurved; disk flowers purple with pubescent teeth; chaffy bracts of involucre nearly equaling disk, truncate and ciliate pubescent at apex: pappus of several short. lacerate squamellae and two awnlike elongated ones; achenes flattened but scarcely winged, from densely long pubescent in the marginal flowers to sparsely pubescent in central ones.

This species seems to be most closely related to the little known *H. microcephala* Gray; from it, however, it is apparently easily distinguished by the very much larger size, the relatively longer ligules, and the different character of the pappus of the new species. Collected at Naturita, August 11, 1914, where it grows in gulches and ravines; no. 591.

CHAENACTIS SCAPOSA Eastwood (*Chamaechaenactis scaposa* [Eastw.] Rydb.).—Nos. 267 and 305 of my collection may best be referred to this species, at least until more is known of the variability of *C. scaposa*. My plants differ principally from the type as described by Miss EASTWOOD in having leaves that are pubescent but not at all hirsute on the upper surface; pappus that is *quite as long* as the achene; and an achene that is appressed long-pubescent rather than villous. The original description does not mention the existence of rows of many small, superficial dark dots on the achene such as the Naturita specimens possess. Collected on gypsiferous hillsides, April 30 and May 15, 1914. It is interesting to note that the flowers of this plant have a peculiar, "heavy" fragrance that reminds one somewhat of *Abronia fragrans*.

UNIVERSITY OF WYOMING
LARAMIE, WYOMING

MORPHOLOGY OF THE LEMNA FROND

FREDERICK H. BLODGETT

(WITH PLATE XIV AND ONE FIGURE)

In the case of structures simplified by reduction, it is sometimes necessary to trace the development of the parts through their immature stages in order to understand the morphology of the mature individuals. This has especial interest when the normal method of reproduction depends upon the development in rapid succession of vegetative propagules, as in the Lemnaceae. The character of such greatly reduced bodies has been variously interpreted, being regarded as stem, leaf, or both fused together, by different writers; while some use a term like "disklike bodies." Since GRAY[1] used *Lemna* as the type of "frondose stem or frond," this name will be used for convenience through the paper. In but few of the articles mentioning the character of the frond has any attempt been made to indicate the basis for the choice of terms, even HEGELMAIER[2] depending largely upon academic argument rather than upon structural details in his discussion of the frond. CALDWELL[3] devoted most of his attention to the development of the flower parts, and did not attempt more than a review of the general character of the frond as presented by others. In the following notes an effort is made to show what structural units contribute to the formation of the frond and the part taken by each in the development of the successive vegetative individuals.

Inception of frond rudiment

The *Lemna* frond is characterized by a sheath or pouch on each side of the base. Within each of these pouches a frond of the next vegetative generation arises, but the two do not develop at the same time. When flowers are produced each one grows in the

[1] GRAY, ASA, Structural botany. New York. 1879 (p. 67).

[2] HEGELMAIER, F., Die Lemnaceen. Leipzig. 1868.

[3] CALDWELL, OTIS W., Life history of *Lemna minor*. BOT. GAZ. **27**:37–66. 1899.

position in which the frond ordinarily grows, although a young frond may later develop within the same pouch. Behind the growing frond or flower there is a bud normally present. The rudiments of this bud appear very early in the development of the frond, in the axil of which it is located. Such a bud is shown in fig. 1. The frond subtending this bud was slightly smaller than that shown on the same scale in fig. 10. The development of the bud involves only elongation at first (figs. 3 and 4), but at an early stage there is a thickening of the basal region. The limitations of space soon force the elongating bud to turn from the nearly erect position to one more or less oblique and finally to the horizontal. This is less rapidly accomplished when the bud lies behind a flower, since in this case the developing seed by its bulk spreads the forward part of the pouch, and leaves immediately behind itself a space in which an erect development of the bud may go on for a considerable time; but even in this case the frond becomes horizontal while still minute.

Differentiation of parts

When the bud is about twice as long as the diameter of its base, the cells of the root initial may be distinguished. They are usually the fourth cell layer from the ventral surface, and develop a cleavage along the contact region of the third and fourth series of cells (figs. 5 and 8). The cells of the fourth layer and deeper develop the root cap and the root structures within the cap; those of the second and third layers are crushed by the elongation of the root tissue; but the outer layer elongates with the forward growth of the root and forms the "root sheath" which commonly persists about the base of the mature root.

At nearly the same time when the root develops there is formed upon the dorsal surface of the bud a slight elevation or mound of cells. This is nearly opposite the group of root initials and marks the origin of the bud for the next frond in the series (fig. 3). Very soon a second mound is visible, close to the insertion of the bud upon the parent tissue, just behind the bud of the daughter frond (figs. 7 and 7*a*). This is the inception of the bud axillary to the frond the development of which is being followed. This bud will

develop but slowly, as compared with the bud anterior to it, which represents the daughter frond; thus when the latter is as shown in fig. 10, the axillary bud is only as shown in fig. 2, or in figs. 6 and 10.

The next stage marks the beginning of the upper wall of the inclosing pouch by which the bud will be protected until large enough to project beyond the pouch margin. This first appears as a sharp projection just anterior to the bud of the daughter frond, as shown in fig. 6. At first the bud and the protective ridge are both in the median region of the young frond, and nearly opposite the root rudiment; but with the continued growth of all parts, the space available for vertical enlargement becomes insufficient and there is a lateral shifting of the dorsal structures. The bud itself becomes divided into two groups of cells, each a potential growing point. The development of the pouch wall is shifted from the position of a covering which is directed posteriorly over the bud, to that of an outgrowth which is directed obliquely backward from either side of the original position.

A further change follows this shifting of the bud. The overgrowing wall is displaced along with the inclosed bud, and thus there are formed two pouches at the base of the frond. The pouch walls, which are first recognized as a slight ridge anterior to the bud rudiment, extend around this as a flattened ring having its insertion along the side of the elongating base or stem of the frond and laterally on the widening frond. At the ends there is but little projecting growth of this ridge, but from the portion just above and below the inclosed bud a considerable development takes place. The pouch walls merge into the body of the frond completely at their insertion.

A vascular connection remains between the two buds after they are separated by the upward growth of the supporting tissue, as is shown in fig. 9. No distinctly vascular elements are developed at this stage, but the elongated cells here shown are their rudiments. This figure also indicates the unequal development of the opposite buds. In *Lemna trisulca* there is less difference in development than in most species, but the general sequence of stages is similar.

Later stages of development

The young frond now has present all the structural units which will be visible in the mature free frond, the later developments being in the manner of enlargement of parts present and the completion of structures already initiated. The two buds in their respective pouches will follow the same stages as the frond developing from an axillary bud, such as is being discussed; while the root shows little of special interest. Two changes which take place, however, should be mentioned; the rapid elongation of the region anterior to the bud-root region, and the thrusting forward of the whole structure by the development of the basal region into a stalk or stipe. In *Lemna* this basal region is attached marginally to the main portion of the frond, but in *Spirodela* it is inserted upon the ventral surface some distance from the edge, and in *Wolffia* it is almost completely lacking.

The axillary bud which was at first upon the dorsal surface of the stipe is forced into a lateral position, as was the case with the frond bud which was divided. In this case the lateral shifting seems to be due to the more rapid growth of the stipe to one side of the median line, resulting in a tipping of the bud and raising its base as the stipe thickens. In the frond this stage is accompanied by the development of air cavities in the anterior region which is elongating rapidly. The individual cells become highly vacuolated, and the stomata are differentiated. The vascular strands appear at this time in the anterior region, which in all essentials assumes the structural characteristics of a foliage leaf.

The vascular cells in the stipe become more clearly defined as a single strand centrally located in the tissues of the connecting structure. This strand ends in a confused group of cells at the point of origin of the vascular tissues of the root, the lateral buds, and the anterior region. This region corresponds to the mat of vascular elements in the base of a bulb as discussed by CHRYSLER[4], and may be considered as locating closely the stem apex of the plant. In bulbs the apical region usually gives rise to a single main bud, with some lateral buds axillary to bud scales. In *Lemna* the

[4] CHRYSLER, M. A., Development of the central cylinder in Araceae and Liliaceae. BOT. GAZ. **38**:161–184. 1904.

terminal bud is divided by vertical pressure and constraint, and appears as a pair of lateral buds except in the initial stages; but the axillary bud is developed in its normal position, behind the foliar outgrowth.

Homologies of the Lemna frond

The *Lemna* frond is a structure of reduced character, especially adapted to vegetative propagation under particular conditions. The two forces which act upon the plant as possible determining factors in the resulting structure are the tension of the water film and the buoyant effect of the liquid. The film tension tends to attenuate in all directions any elastic structure floating upon it, while the upward thrust of buoyancy tends to lift any deep-lying portion into the same plane with the thinner parts. The thin frond responds to the conditions imposed, and may be considered as a result of the forces involved. The roots act as drags or brakes against too free movement in the water, and to return the dorsal surface to the air after the frond has been immersed by any sudden shock, the drag of the projecting roots tending to orient the frond as it rises to the surface by its buoyancy. Inverted plants of *Spirodela* will thus orient themselves in a 3-inch dish of water, but complete inversion would but rarely occur under natural conditions.

The runner of *Erythronium* is a structure also of reduced nature and also developed to meet particular conditions. In this case the forces involved are the outward thrust of growing tissues and the resistance of the surrounding soil. The root type of structure is manifestly best fitted to this condition, having the region of elongation close to the apex of the structure. An investigation of the development of these runners[5] showed that the zone of elongation is so located, and that the component parts of the runner are adapted to the conditions and factors present, namely, the offsetting of propagules, and their movement through soil. A longitudinal section of a runner is shown in text fig. 1 (I). The bud scale forming the anterior wall of the hollow tip elongates, as do leaves, by basal growth; the axial portion of the runner which thrusts the terminal bud forward elongates by growth near the tip. The two

[5] BLODGETT, F. H., Origin and development of bulbs in *Erythronium*. BOT. GAZ. 50:340–372. 1910.

growing regions harmonize completely, and the inclosed bud is thrust forward through the soil, with no serious injury from antagonistic growth forces. The pointed cylinder meets the conditions of progress through a solid medium as does the flat frond for floating upon a fluid surface.

The *Lemna* frond may be closely compared to the *Erythronium* runner in structural details. If the outer scale of the terminal

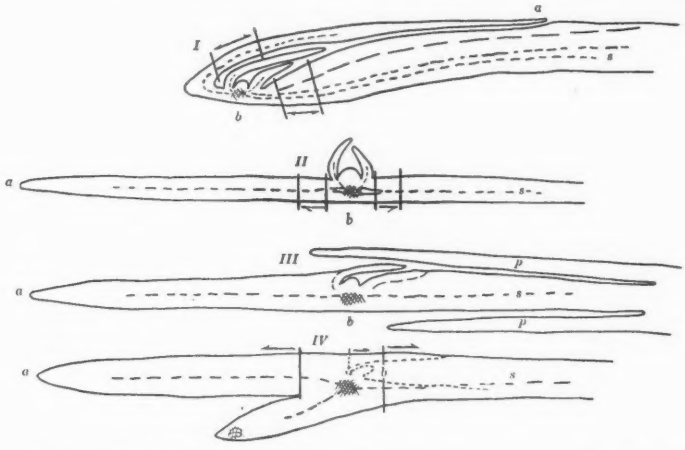


FIG. 1.—Homology of *Lemna* frond and *Erythronium* runner: *a*, tip of first leaf; *b*, stem apex of bud; *s*, shoot axis; *pp*, walls of pouch.

bud of the runner could be turned forward on its base as a hinge, a structure would result of the character shown at II in the text figure. This has a terminal foliar region, a central one from which a dorsal bud and ventral root originate, and a basal stem or cauline region,[6] but the bud is erect. The growing regions are still at the base of the foliar and near the tip of the cauline portions of the whole. If this were to be formed within a vertically restricted space, the dorsal bud would be flattened, and probably somewhat

[6] These correspond respectively to the "upper internode," the "node," and the "basal internode" of CALDWELL (*op. cit.*, p. 43), and supports his use of the word "shoot"; but the shoot consists of a terminal leaf, a node with bud tissues, and a stem, rather than of an "undifferentiated upper internode" and specially modified "basal and nodal regions."

fused to adjacent parts (as in III of the text figure), as is the case on the posterior surface of the bud scale in the runner bud shown at I. The vertical thickening of the tissues at the insertion of the bud would tend to split the meristem group of cells so that a pair of lateral buds would finally result, as in the *Lemna* structure.

The protective inner scale of the bud, which is represented in diagram III as bent backward under the overlying pouch wall, in the *Lemna* frond shown at IV, is fused with the posterior margin of the foliar tissues of the frond, and laterally to the side of the stem. This pouch wall arises as a ring about the base of the bud which it will finally inclose, and is therefore homologous with the development of the scale leaf about the stem tip of a bulb, or a bud of the *Erythronium* type. Under the adjustment to space and pressure, the ring is flattened to a narrow eclipse, and is divided by the same conditions as those which divide the bud within.

In the region immediately below the stem apex in bulbs, and the corresponding point in *Lemna*, the root initials are developed; this region is indicated in the diagrams by cross-hatching, adjacent to *b* in I, II, III. In *Lemna* only one root is regularly developed, but in *Spirodela* several are formed. The activity of the adjacent apical meristem evidently continues for a considerable period, allowing for the successive formation and development of new fronds within the same parent pouch. The old fronds are released by the separation of the cells close to the insertion of the stem upon the parent tissues, apparently by the breaking down of the middle lamella of a band transverse to the stem. The stump left in such a case is seen at *s* in fig. 2. By the enlargement of the axillary bud this stump will be obliterated before the new frond is far advanced.

Conclusions

The *Lemna* frond is a propagative structure consisting of a terminal leaf; a bud inclosed by a flattened bud scale, the base of which is fused to the base of the leaf and laterally to the stem; and an apical region from which new fronds are developed. Two buds are formed through the splitting of a single bud rudiment by vertical pressure during early stages of growth. The frond meets the conditions of a floating habitat in which the tension of the

surface film apparently is an active factor. Through the lack of space for vertical succession, the several outgrowths from the apical region are liberated as a horizontal series, the overlapping of successive individuals forming an element of confusion in an examination of their structure.

TEXAS AGRICULTURAL EXPERIMENT STATION
COLLEGE STATION, TEXAS

EXPLANATION OF PLATE XIV

FIGS. 1, 2.—Early stages in formation of axillary buds: magnification, indicated by scale of 0.05 mm., uniform for all figures except fig. 9, which has its special scale indicated.

FIG. 3.—Median section of older bud: *b*, daughter bud rudiment; *a*, axillary bud.

FIG. 4.—Similar section at next stage: buds displaced by thickening of base; 4*a*, details of root from adjacent section.

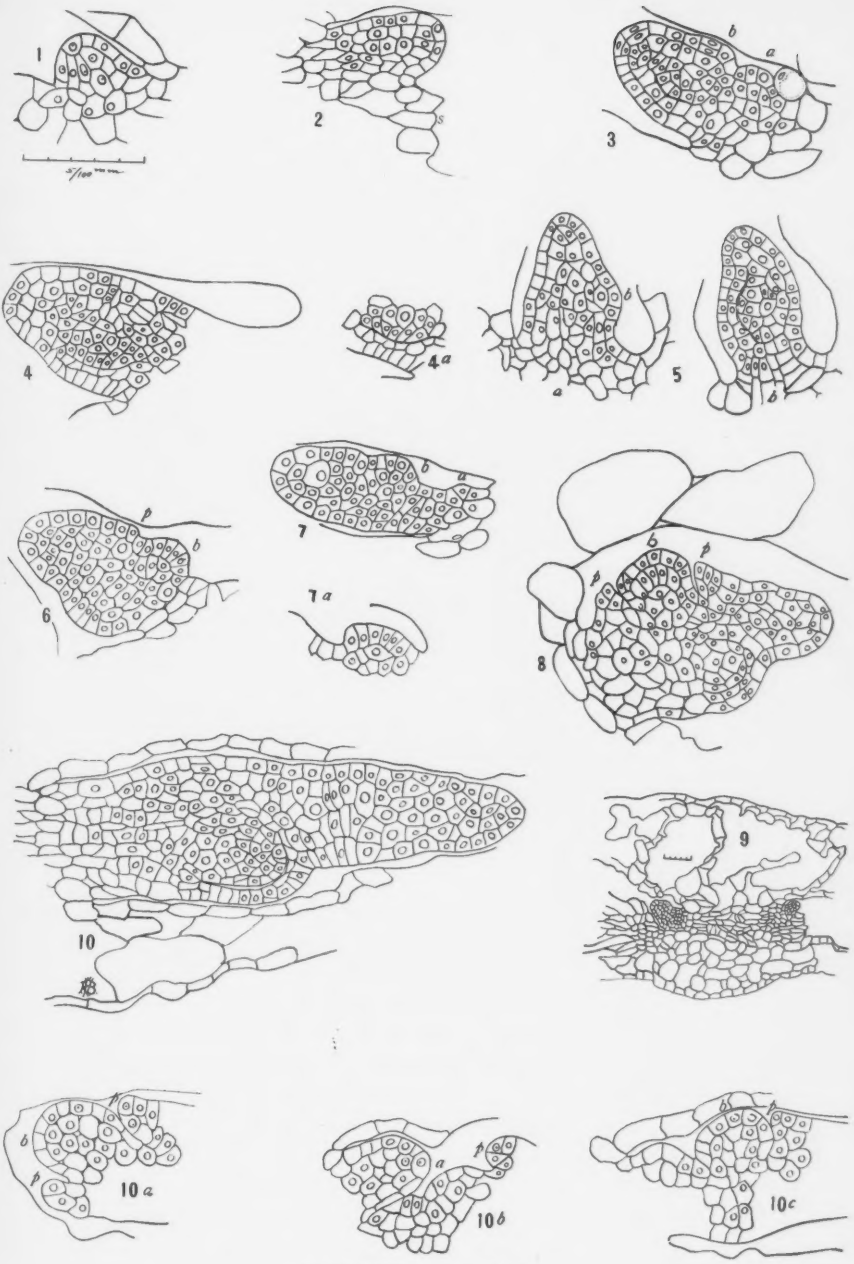
FIG. 5.—Adjacent sections of bud in axil of nearly grown seed (to left), showing daughter bud and root regions; axillary bud not yet defined; intermediate in age between figs. 2 and 3.

FIGS. 6, 7.—Daughter buds from opposite sides of median section shown in fig. 4; 7*a*, axillary bud the edge of which shows at *a* in fig. 7.

FIG. 8.—Sagittal section of frond, showing origin of inclosing scale leaf or pouch (*p*,*p*) about daughter bud (*b*); below the root is pushing out, and to the right the leaf is elongating.

FIG. 9.—Transverse section through apical region of frond, showing displaced halves of daughter bud, now unequally developed, and connecting cells; compare figs. 2 and 3; low power.

FIG. 10.—Young frond in sagittal section: leaf forms right half; basal buds partly inclosed by bud scale or pouch (at 10*a* and 10*c*); axillary bud at 10*b*, with edge of buds (*a* and *c*) and of pouch wall (*p*).



BLODGETT on LEMNA

NOTES ON NORTH AMERICAN WILLOWS. III

CARLETON R. BALL

The first[1] of a projected series of papers under this title was published in this journal in November 1905. Since that date, the writer's contributions on Salices have been confined to treatment of the genus for two floras covering widely different sections of the country, namely, the Rocky Mountains[2] and the Central Atlantic states.[3] In the ten-year period, however, thousands of specimens have been determined for collectors and herbaria, extensive field studies and collections have been made, and a considerable fund of data accumulated, some of which are presented in the third paper[4] of this series and in the following paragraphs.

1. The willows of the Black Hills

The first list of the plants of this district, of which the writer has knowledge, was published by RYDBERG[5] in 1896. He collected at some 20 points in the Black Hills, during the period from May 27 to August 18, 1892, visiting some places twice. Four species of *Salix* are recorded (p. 523): *S. fluviatilis* Nutt., *S. cordata* Muhl., *S. discolor* Muhl., and *S. Bebbiana* Sarg. Of these, *S. fluviatilis* is listed only from Rochford; *S. cordata* only from Custer and Lead; *S. discolor* only from Custer; while *S. Bebbiana* was collected at 5 points. No data on frequency of occurrence are given for the willows, though such notes are given commonly throughout the paper. It is probable, however, that the three species first named were met with at points other than where collections were made.

[1] BALL, CARLETON R., Notes on North American willows. I. BOT. GAZ. **40**:376–380. *pls. 12, 13.* 1905.

[2] ———, *Salix* in COULTER and NELSON, New Manual Rocky Mt. Bot. pp. 128–139. 1909.

[3] ———, *Salix* in TIDESTROM, Elysium Marianum **3**:19–37. *pls. 4–9.* 1910.

[4] ———, Notes on North American willows. II. BOT. GAZ. **60**:45–54. *figs. 3.* 1915.

[5] RYDBERG, P. A., Flora of the Black Hills of South Dakota. Contrib. U.S. Nat. Herb. **3**:463–536. 1896.

While the Black Hills have always been recognized as belonging to the Rocky Mountain system, and as having a flora allied to that of the Rocky Mountain region, it will be noted that the four willows reported by RYDBERG are all eastern species. They range from the North Atlantic and the New England states westward across the great interior valley. *S. Bebbiana* extends commonly and *S. fluviatilis* less commonly into the Rocky Mountain region itself, but primarily they are eastern species. The question of the identification of the specimens collected is not raised at this point.

Another list which includes the Black Hills district was published by WILLIAMS,[6] presumably in 1895. The willows of the state, 10 in number, include the 4 of RYDBERG's Black Hills list and also 3 others credited to the Black Hills. It does not appear from this publication that herbarium specimens of all the species were obtained. WILLIAMS refers frequently to RYDBERG's collections from the Black Hills and was doubtless familiar with a manuscript copy of his list. The 3 additional species credited to the Black Hills are as follows:

"18. YELLOW WILLOW (*Salix flavescens* Nutt.).—A shrub found in the Black Hills. It may be recognized by the nearly entire leaves which are downy, or smooth and dull green above, and pale with a rufous pubescence beneath" (p. 106). No reference is made to any collections.

"19. HAIRY WILLOW (*Salix glauca villosa* Anders.).—. . . . Found at various places in the Black Hills and on the Yellow Bank in Grant County" (p. 106).

"20. PRAIRIE WILLOW (*Salix humilis* Marsh.).—A small shrub reported from the Black Hills by Mr. RYDBERG" (p. 107). In no case is herbarium material cited. Again the question of identification is waived for the time.

A third list covering the territory of the Black Hills in South Dakota was published by SAUNDERS.[7] The striking thing about this list is that it does not include two of the three additional willows credited to the Black Hills by WILLIAMS, namely, *S. flavescens* and *S. glauca villosa*. It does list *S. humilis*, but as occurring only

[6] WILLIAMS, T. A., Native trees and shrubs. S.D. Exper. Sta. Bull. 43. 1895 (pp. 105–107).

[7] SAUNDERS, D. A., Ferns and flowering plants of South Dakota. S.D. Exper. Sta. Bull. 64. 1899 (pp. 134, 135).

in the eastern part of the state. The reasons for omitting these three willows can be guessed only. Either specimens were not found in the herbaria examined or they proved to belong to other species than those listed. It is a fair guess that *S. humilis* was included by WILLIAMS on the strength of RYDBERG's[8] identification of his no. 1019 from Rochford, of which he says:

> A few specimens with thick leaves as in *S. humilis* and *S. tristis* were collected at Rochford, altitude 1700 m., July 12. Even these have been referred to *S. rostrata* by Mr. BEBB (no. 1019).

At any rate, the list of Black Hills willows was again reduced to four species. So far as publications are concerned, the number seems still to rest there, though in 1912 PERISHO and VISHER[9] reported *Salix lutea*, a western species, as being "fairly common along brooks" in Washington County, which adjoins the Black Hills on the southeast.

On September 19, 1908, the writer studied and collected the willows occurring along Whitewood Creek from the railroad station upstream for a mile or more. Five species were found. Two of them, *S. lutea* Nutt. and *S. prinoides* Pursh, were very common in the floodplain of the stream. Of each of the remaining three, *S. Fendleriana* Anderss., *S. Scouleriana* Barr., and *S. Bebbiana* Sarg., only a single plant was found. All three were obtained in the edge of the city. An examination of RYDBERG's specimens showed that *S. lutea* was the species reported as *S. cordata* by him, while *S. prinoides* was the basis of his record for *S. discolor*. The finding of *S. Scouleriana* bears out the statement quoted from WILLIAMS under *S. flavescens*, which is a synonym. This is the first recorded collection of *S. Fendleriana* in the Black Hills. It occurs commonly in the canyons of the Rocky Mountains and westward at elevations of 5000 feet and over. The number of species was now increased to six.

Collections of *S. amygdaloides*, *S. fluviatilis*, and *S. cordata* were made also at Bellefourche on the same date, but as Belle-

[8] *Op. cit.*, p. 523.

[9] PERISHO, E. C., and VISHER, S. S., The geography, geology, and biology of south central South Dakota. State Geol. and Biol. Survey Bull. 5. p. 90. 1912.

fourche properly is not in the Black Hills, *S. amygdaloides* is not added.

On September 14, 1910, the writer again visited Deadwood for a short time and studied the willows found on the northern flank of the White Rocks, principally around a spring lying just below the wagon road. Here *S. Bebbiana* and *S. prinoides* were found abundantly and *S. lutea* less commonly on the seepy slope below the spring. Two or three stunted plants of *S. Scouleriana* occurred in dry ground just across the road above the spring.

On August 23, 1913, the writer collected *S. lutea* at the railroad station at Mystic, and *S. Bebbiana* and *S. Scouleriana* again at the spring north of the White Rocks at Deadwood. All these collections are cited under the respective species at the end of this article.[10]

In the spring and summer of 1910, Mr. JOHN MURDOCH, JR., of the United States Forest Service, collected an extensive series of specimens in various parts of the Black Hills. A portion of the series was sent to the writer for identification through the United States Forest Service, on April 1, 1913. Seventeen numbers were flowering specimens of *S. prinoides* and *S. Bebbiana*, one was *S. fluviatilis*, and one proved to be a young pistillate specimen of *S. Nelsonii* Ball, a rather rare member of § PHYLICIFOLIAE, known previously from the mountains of Colorado and Wyoming. A more complete set from the private herbarium of the collector was submitted to the writer by Dr. GEO. B. SUDWORTH, dendrologist of the Forest Service, on January 14, 1915. It was found to contain, besides numerous specimens of *S. prinoides* and *S. Bebbiana*, the specimen of *S. Nelsonii* (no. 4039) mentioned above, collected at Redfern Plantation no. 2, and also a specimen bearing mature foliage (no. 4233), collected at the same place in July.

[10] Since this paper was prepared, the writer has seen a series of short papers by VISHER, entitled Additions to the flora of the Black Hills of South Dakota (Torreya **9**:186–188. 1909; Muhlenbergia **8**:135–137. 1913; *ibid.* **9**:33–39. 1913). In the first paper he records *S. Scouleriana* as "Frequent, forming trees, in deep woods, well up on Custer's Peak." The collection was made in August 1908, just a month before it was collected by the writer at Deadwood.

In one of another series of papers (Additions to the flora of South Dakota. II. Muhlenbergia **9**:69–77. 1913) VISHER reports *S. Fendleriana* as "Common in boggy soil in the forest reserves and along the Little Missouri River" of Harding County, north of the Black Hills, in the northwest corner of the state.

There was also one specimen each of *S. amygdaloides* and *S. lutea*. Very interesting also were a specimen of *S. Fendleriana* (no. 4375), with mature foliage, from Rochford; three foliage specimens of *S. Scouleriana*, also from Redfern Plantation no. 2; a foliage specimen of *S. Geyeriana* (no. 4367) from Bear Butte Creek; and finally a single collection of what is probably *S. monticola* Bebb (no. 4374) from South Rapid Creek, at an elevation of 6100 feet. By this collection four species, *S. amygdaloides*, *S. Geyeriana*, *S. monticola*, and *S. Nelsonii*, not previously reported, were added to the flora of the Black Hills. All except the first are strictly plants of the western mountains. The specimen identified as *S. monticola*, which consists of autumn-colored foliage collected September 16, is certainly not referable to either *S. cordata* or *S. lutea*, and with equal certainty is a species not previously recorded for the Black Hills. Two of the sheets of *S. Scouleriana* show only small but mature leaves, suggesting a dwarfed habit, and the data indicate that they come from dry situations.

In the early spring of 1913, some fragmentary specimens collected in 1912 by Messrs. T. C. SETZER and N. E. PETERSEN, of the Merritt Ranger Station in the Black Hills Forest Reserve, were sent the writer for identification. On examination these were found to include such Rocky Mountain species as *S. glaucops* Anderss., *S. chlorophylla* Anderss., *S. Nelsonii* Ball, and *S. Geyeriana* Anderss. Of these, the first two had not been collected previously in the Black Hills. At the request of the writer, Mr. W. H. LAMB, of the Forest Service, kindly made immediate arrangements to have better material collected in 1913. As a result, there were received for identification, on January 21, 1914, another series of these same species. Careful examination confirmed the previous determinations and hence added two more species to the list of those known to be native to the Black Hills. This list now contains 12 species, all native. Three out of the 12, namely, *S. amygdaloides*, *S. fluviatilis*, and *S. Bebbiana*, are found nearly across the continent. One, *S. prinoides*, is an eastern species finding here nearly the western limit of its range. The 8 others are habitants of the Rocky Mountains and westward, which find the eastern limits of their ranges in the Black Hills.

KEY TO THE SPECIES

Capsule glabrous
Scales yellow
Leaves large, lanceolate, serrate; stamens 3–5
Leaves glaucous beneath ..... 1. *S. amygdaloides.*
Leaves green beneath ..... 2. *S. Fendleriana.*
Leaves linear; stamens 2
Leaves green beneath, remotely denticulate ..... 3. *S. fluviatilis.*
Scales brownish to black
Aments short-peduncled; twigs yellow; style less than 0.5 mm. 4. *S. lutea.*
Aments sessile; twigs reddish; style 1 mm. or over ..... 5. *S. monticola.*
Capsule hairy
Style long; scales usually black, pilose
Aments peduncled; leaves gray pubescent above ..... 6. *S. glaucops.*
Aments sessile; leaves bright green above, glabrous
Leaves broadly elliptical or oval ..... 7. *S. chlorophylla.*
Leaves narrowly elliptical ..... 8. *S. Nelsonii.*
Style short or none
Scales obovate, black, pilose; aments sessile
Leaves elliptical, undulate-crenate, glabrous ..... 9. *S. prinoides.*
Leaves obovate, entire, often pubescent beneath ... 10. *S. Scouleriana.*
Scales linear-oblong, yellow or the tip dark; aments short-pedunculate
Leaves elliptic-obovate; pedicels long ..... 11. *S. Bebbiana.*
Leaves linear-oblanceolate; pedicels short ..... 12. *S. Geyeriana.*

SPECIMENS EXAMINED

The following specimens have been examined by the writer. Their present location in herbaria, so far as known, is indicated by the letters following the collection numbers. The key to these letters is: B, Herb. C. R. BALL; C, Herb. Canada Geol. Survey; F, Herb. U.S. Forest Service; G, Gray Herbarium; I, Herb. Iowa State College; M, Herb. JOHN MURDOCH, Jr.; N, U.S. National Herbarium; R, Rocky Mountain Herbarium.

1. *Salix amygdaloides* Anderss.

BELLEFOURCHE.—Floodplain of Bellefourche River, *Ball* (1341, B, C, N, R) 1908; (1697, B; 1699, B) 1910.

DEADWOOD.—Whitewood Creek below Smelter, alt. 1370 m., *Murdoch* (4322, M) 1910.

2. *S. Fendleriana* Anderss.

DEADWOOD.—Bank of Whitewood Creek, south edge of town, alt. about 1400 m., *Ball* (1356, B, N, R) 1908.

ROCHFORD.—Alt. 1585 m., *Murdoch* (4375, M) 1910.

3. *S. fluviatilis* Nutt.

BELLEFOURCHE.—Floodplain of the Bellefourche River, *Ball* (1340, B, C, N, R; 1344, B, N) 1908; (1696, B, N, R) 1910.

ROCHFORD.—Alt. 1675 m., *Rydberg* (1020, N) July 11, 1892.

SAVOY.—Creek banks, alt. 1525 m., *Murdoch* (4166, F, M) 1910.

4. *S. lutea* Nutt.

BELLEFOURCHE.—Floodplain of Bellefourche River, *Ball* (1694, B, N; 1695, B, C, N, R), foliage, 1910.

CUSTER.—Alt. 1690 m., *Rydberg* (1015, N) June 4, 1892.

DEADWOOD.—Floodplain of Whitewood Creek, alt. about 1400 m., *Ball* (1352, B, C, G, N, R; 1354, B), foliage, 1908; spring below White Rock, alt. about 1460 m., *Ball*, 1686, B, C, I, N, R), foliage, 1910.

FT. MEADE.—*Forwood*, flowers, May 1, 1887.

LEAD.—Alt. 1675 m., *Rydberg* (1017, N), foliage, July 7, 1892.

MYSTIC.—At railroad station, alt. 1460 m., *Ball* (1827, B, N), foliage, 1913.

REDFERN.—Plantation No. 2, alt. 1675 m., *Murdoch* (4038, M), pistillate flowers, May 13, 1910.

STURGIS.—Alt. 1065 m., *Carr* (344, B; 345, B), staminate and pistillate flowers, May 17, 1912.

5. *S. monticola(?)* Bebb

SOUTH RAPID CREEK.—Alt. 1860 m., *Murdoch* (4374, M), foliage, September 16, 1910.

6. *S. glaucops* Anderss.

MERRITT.—Ranger Station, headwaters of Jim Creek, Sec. 8, Tp. 2 N., Range 5 E., alt. about 1525 m., *Setzer* (F) 1912; *Setzer* (2, B), staminate, May 6, 1913.

7. *S. chlorophylla* Anderss.

MERRITT.—Ranger Station, headwaters of Jim Creek, Sec. 8, Tp. 2 N., R. 5 E., alt. 1525 m., *Setzer* (F) 1912; *Setzer* (1, B), staminate, May 6, and foliage, June 2, 1913.

8. *S. Nelsonii* Ball

MERRITT.—Ranger Station, headwaters of Jim Creek, Sec. 8, Tp. 2 N., R. 5 E., alt. 1525 m., *Setzer* (F) 1912; *Setzer* (3, B), pistillate, May 6, and foliage, June 2, 1913.

REDFERN.—Plantation no. 2, alt. 1710 m., *Murdoch* (4039, B, M), pistillate, May 13, 1910; *Murdoch* (4233, M), foliage, July 13, 1910.

9. *S. prinoides* Pursh

BEAR BUTTE CREEK and vicinity.—Mostly 6–8 miles southeast from Deadwood, and about the same distance northeast from Custer Peak; alt. 1525–1650 m., *Murdoch* (4002–4015 inclusive, F, M), staminate and pistillate flowers, April 7–10, 1910; (4366, 4369, both M), foliage, September 8, 1910.

CUSTER.—Alt. 1675 m., *Rydberg* (1014, N), staminate and pistillate flowers, May 31, 1892.

CUSTER PEAK.—About 3 miles E., alt. 1710 m., *Murdoch* (4001, F, M) staminate flowers, April 7, 1910.

DEADWOOD.—Floodplain of Whitewood Creek, alt. about 1400 m., *Ball* (1349, B, N, R; 1353, B, C, G, N, R; 1355, B, N, R), foliage, 1908; at spring north of White Rock, alt. about 1460 m., *Ball* (1681, B, N, R; 1684 and 1685, B, C, I, N, R), foliage, 1910.

DUMONT.—Alluvium, Thompson's Bridge, alt. 1830 m., *Murdoch* (4267, M), foliage, August 4, 1910.

STURGIS.—*Carr* (308, B), flowers, May 17, 1912.

10. *S. Scouleriana* Barratt

DEADWOOD.—Bank of Whitewood Creek, alt. about 1400 m., *Ball* (1350, B, C, G, N, R), foliage, 1908; at spring below White Rock, alt. about 1460 m., *Ball* (1683 and 1687, B, C, I, N, R), foliage, 1910, (1832 and 1833, B, N), foliage, 1913.

REDFERN.—Plantation no. 2, alt. 1740 m., *Murdoch* (4220, 4221, 4237, all M), foliage, July 13, 1910.

11. *S. Bebbiana* Sargent

BEAR BUTTE CREEK.—About 5–8 miles S.E. of Deadwood, alt. 1555 m. *Murdoch* (4010, in part, and 4022*a*, F, M), flowers, April 10–27, 1910; (4368, M), foliage, September 8, 1910.

CUSTER.—Alt. 1675 m., *Rydberg* (1012, N; 1013, N), May 31 and June 6, 1892.

CUSTER PEAK.—About 3 miles E., alt. 1710 m., *Murdoch* (4365, M), foliage, September 8, 1910.

ELK CANON.—Alt. 1525 m., *Rydberg* (1016, N), June 2, 1892.

DEADWOOD.—Floodplain of Whitewood Creek, *Ball* (1351, B, N), foliage, 1908; (1680 and 1682, both B, C, N, R), foliage, 1910; (1829, B, N; 1830 [pistillate], B, N; 1831 [pistillate], B), foliage, 1913.

HOT SPRINGS.—Alt. 1100 m., *Rydberg* (1018, N), August 9, 1892.

REDFERN.—Plantation no. 2, alt. 1710–1740 m., *Murdoch* (4036 and 4037, F, M), flowers, May 13, 1910; (4219 and 4232, M), foliage, July 13, 1910.

ROCHFORD.—Alt. 1675–1830 m., *Rydberg* (1019, N), foliage, July 12, 1892.

12. *S. Geyeriana* Anderss.

BEAR BUTTE CREEK.—Alluvium, Sec. 19, Tp. 4 N., R. 4 E., alt. 1645 m., *Murdoch* (4367, M), foliage, September 9, 1910.

MERRITT.—Ranger Station, headwaters of Jim Creek, Sec. 8, Tp. 2 N., R. 5 E., alt. 1525 m., *Setzer* (F) 1912; (4, B), staminate flowers, May 6; leaves, June 2, 1913.

U.S. DEPARTMENT OF AGRICULTURE
BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.

ON THE OCCURRENCE AND SIGNIFICANCE OF "BARS" OR "RIMS" OF SANIO IN THE CYCADS

H. B. SIFTON

(WITH PLATE XV)

The "bars" or "rims" of Sanio were probably first figured by GÖPPERT (**2**) in 1849, as has been pointed out by Miss GERRY (**1**). SANIO (**7**), however, did not, as she states, claim to be the discoverer of them, although he was the first to describe them at all adequately, and hence they have borne his name. In studying the development of the wood cells in *Pinus silvestris*, he found thickened rings, complete or incomplete, which grew up around thin spots in the cellulose primary walls. The thin areas he designated as the "Primordialtüpfeln," and on these bordered pits were formed. The thickened rims, or "Umrissen," of these areas are the structures that have until recently been known as the "bars of Sanio." In 1913 GROOM and RUSHTON (**3**) applied the name "rims" to them. This term is descriptive of SANIO'S theory of their method of formation and serves to distinguish them from trabeculae.

Until the last few years they did not come into much prominence, but in several recent papers they have been considered of great importance. Miss GERRY, in the paper mentioned above, gave a comprehensive account of their distribution in the conifers. She found them in all the members of this group except the araucarians, whose wood structure could in this way be distinguished from the other forms. She also suggests the extension of this distinction to the fossil forms, as the following sentence indicates: "The distribution of the bars of Sanio . . . . establishes a constant and useful diagnostic character in the determination of fossil woods" (p. 122). She would associate all the fossil conifers which have the "rims" with the Abietineae, and those which do not exhibit them with the Araucarineae, regardless of other characteristics of their tracheary structure.

Miss GERRY's suggestion has been followed in a number of recent articles on fossil plants. Miss HOLDEN (**4, 5**) has used it in several papers. Her statement of the importance of the "rims" is even stronger than that of Miss GERRY, making their occurrence "by far the most reliable criterion for diagnosing coniferous woods" (**5,** p. 252). Again, in another paper she speaks still more strongly: "Comparative examination of living and fossil forms leads to the rejection of all criteria except cellulose bars of Sanio as an infallible test for tribal affinities" (**4,** p. 544).

JEFFREY (**6**) has also attached much weight to it, especially since his discovery in 1912 of what he considers a vestigial bar in the araucarian cone axis. His conclusion from its presence here is stated as follows: "*Agathis* and *Araucaria* have obviously come from ancestors which, in accordance with the accepted principles of comparative anatomy, had . . . . bars of Sanio in their tracheids" (p. 548). The phylogenetic significance which he attached to its occurrence in this position figures prominently in his theory of the abietinean ancestry of the Araucarineae. Recognizing that his conclusion from its occurrence in the araucarian cone axis would be invalidated if the same condition were found to occur in similar regions of forms such as the cycads, JEFFREY examined the primitive regions of *Cycas*, *Zamia*, and *Ginkgo*, but found no evidence of its presence. He was therefore doubly assured of its meaning in the araucarians.

In connection with some investigations of the wood structure in the cycads, I have found a "bar"[1] of Sanio entirely similar to that in the araucarians, and since this discovery has such an important bearing on the theories above stated, it has been thought advisable to publish it separately.

The material in which the "bars" were found is from the petiole of *Cycas revoluta*. The sections of this, as well as all the others figured in the plate, were stained in the usual way with a double stain of Haidenhain's iron-hematoxylin and safranin. The bars thus appear in the sections as dark bands on a red background and stand out as distinctly as in the photographs.

[1] The name "bar" has been retained in this paper for the structure as found in *Araucaria* and *Cycas*, for considerations to be stated later.

Fig. 1 is from a section of the vascular bundle of the *Cycas* petiole cut so as to show the tangential walls of the tracheids of the primary wood. Spiral and scalariform elements of the xylem are shown at the left center of the figure, while the two transitional tracheids to the right of these exhibit the border-pitted condition typical of the region. Crossed pores can be seen in several of the pits, showing that the walls are not adjacent to parenchyma, but separate the lumina of two tracheids. The dark lines observable between the pits are thickenings in the primary wall and constitute the "bars of Sanio."

Fig. 3 is a portion of fig. 1 at a higher magnification and shows the part of the two border-pitted tracheids represented between (*a*) and (*b*) on that figure. The knife has passed diagonally through the wall of the left tracheid, leaving only a portion of one of the secondary layers, with parts of the primary attached to it in the upper region. In this area the bars of Sanio come out clearly, especially in connection with the fourth to the seventh pits from the top of the series.

The forking which has been mentioned by JEFFREY as occurring in the bars which he found in the araucarian cone axis is indicated here. An extreme instance of this can be seen between the fifth and sixth pits from the top. These pits are farther apart than is usual, and the bar instead of having merely its ends forked is cleft throughout its length, half clinging to each pit, as a definite "rim" of Sanio. This seems to indicate a double origin for the bars, suggesting that each may have its origin in two "rims," formed in the manner indicated by SANIO, one in connection with each pit. In the tracheid at the right, the bars are very plainly visible, though the forking is not so evident.

The "rims" which form the bars in *Cycas* are characterized especially by two features. They invariably cling closely to the borders of the pits, and they are short, never reaching across the entire width of a tracheid, but only across that of a single pit. These are the two characteristics mentioned by THOMSON (8) as distinguishing the type of bar found in the Araucarineae from that in the members of the Abietineae.

Fig. 4 is from the cone axis of *Araucaria Bidwilli* and shows the structures referred to by JEFFREY as occurring there. Here again, in the upper part of the figure, the wall has been partly cut away. The photograph is at the same magnification as fig. 3, and on comparison it will be seen that the characters of the bars in the two are identical. Here also they invariably cling closely to the pit borders and extend only across the width of a single pit. In almost every case the forking is plainly seen. A very favorable place for observing this is in the left hand tracheid at about the center of the figure. Near the top of this tracheid and in the majority of cases in the other two, the pits are so closely approximated horizontally, owing to the multiseriate character of the pitting, that the ends of the forks of adjacent bars meet, so that only more or less trapeziform lighter areas are seen between them. At one place, indeed, below the center of the middle tracheid, even this is not shown. It is quite evident, however, that this is not a case of a single bar passing across two pits, but of the application of two bars to each other, end to end. This is more evident from the section than from the photograph, for in it a light area similar to those shown in the series above, though not so pronounced, is visible.

There are, therefore, bars in the primitive region of *Cycas* exactly comparable to those in the araucarian cone axis. This, as JEFFREY recognized, makes their presence in the araucarian cone axis valueless as an argument in favor of abietinean ancestry of the Araucarineae. Indeed, the evidence of a reverse relationship is apparent from a study of the characters of the "bar" in the different regions of the Abietineae themselves.

The typical condition in old stem wood of the Abietineae is illustrated in fig. 2, a radial section of *Abies amabilis*. Where the pits are closely approximated in vertical series, as for example in the left tracheid of the figure or in the lower part of the tracheid to the right, the "rims" unite to form bars. Where the pits are scattered, as in the central part of the middle tracheid, each "rim" stands by itself. In neither case, however, are the "rims" closely appressed to the pit borders, as is always true in the araucarian and cycad form. They are not, moreover, limited in length

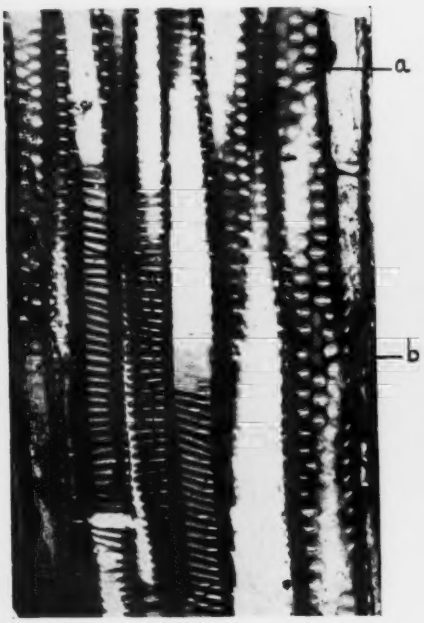
to the width of a single pit. This is especially striking in the tracheid to the left, where opposition and even multiseriate pitting is in evidence.

In fig. 5 is shown a section of the wood of the cone axis of *Pinus resinosa* near the primary xylem. It was difficult to get a good photograph on account of the irregularities in the tracheid walls. The figure illustrates the type of "rim" found in this region. The structures usually adhere closely to the pit borders, appearing as dark arcs, and not extending beyond the pits. One long bar, however, is shown just below the four pits at the top of the tracheid, passing across nearly the width of two pits, and thus presenting to some extent the appearance of the bar found in specialized abietinean wood. Such structures are exceptional.

Fig. 6, from a radial section of the root of *Pinus Strobus*, shows a condition quite often found in the secondary wood close to the primary. Although the pits are large, the "rims" of Sanio, which are plainly in evidence, present the same characters as those of fig. 5. As one passes out from this region into the outer root wood, transitions are seen between this rim and the type shown in fig. 2. The rims become more and more separated from the pits. They also elongate and exhibit a tendency to fusion. The mature bar is thus often a complex structure and very specialized in character. Similar transitions are seen in the cone axis in passing outward from the center.

It is generally admitted that root and cone axis retain ancestral characters longer than the stem or branches, and the earliest formed parts of these are also accounted more primitive than the parts formed when the plant is older. If in this case the accepted reasoning holds true, the Abietineae must have been derived from ancestors which had bars of the araucarian or cycadean type.

The discovery of "bars" or "rims" of Sanio in the primitive region of the cycads must either nullify their value as evidence of the derivation of the araucarians from the Abietineae, or indicate that the Abietineae are also ancestral to the cycads, a position which can scarcely be assumed. Moreover, the study of the rims in different regions of the Abietineae indicates that the ancestral type of bar, as found in the cycads and araucarians, has become



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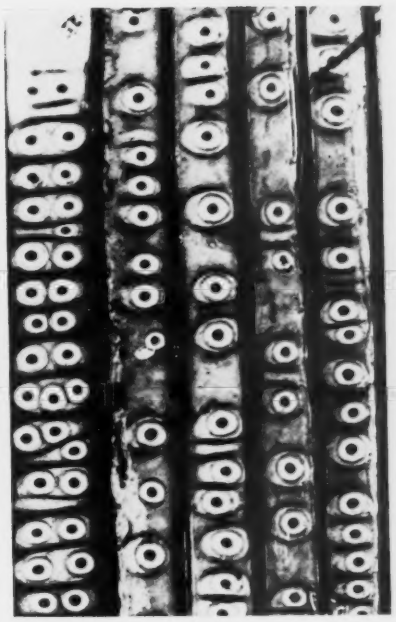

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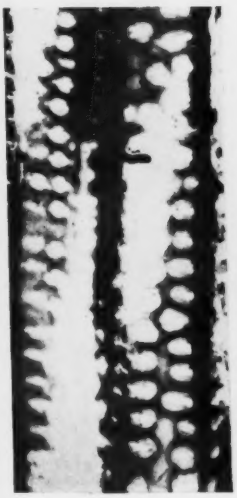

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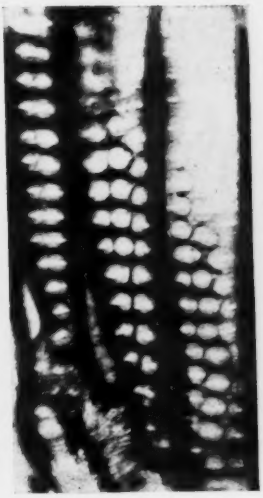

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SIFTON on BARS OF SANIO

specialized in the higher conifers, the original type persisting only in the primitive regions, the cone axis and the root. Thus the discovery of the bar in the cycads has, besides confirming the primitive character of this structure in the araucarians, made apparent its significance in the different regions of the Abietineae.

This work has been carried on at the suggestion of Professor R. B. THOMSON, to whom I wish to express my indebtedness for his constant assistance.

UNIVERSITY OF TORONTO

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EXPLANATION OF PLATE XV

FIG. 1.—*Cycas revoluta:* petiole; tangential section of primary wood; ×225.

FIG. 2.—*Abies amabilis:* radial section of secondary wood of stem, showing "rims" and "bars" of Sanio; ×225.

FIG. 3.—*Cycas revoluta:* a portion of fig. 1 more highly magnified; ×445.

FIG. 4.—*Araucaria Bidwilli:* radial section of secondary wood of seed cone axis, showing "bars" of Sanio; ×445.

FIG. 5.—*Pinus resinosa:* radial section of secondary wood of seed cone axis close to the primary wood, showing primitive "rims" of Sanio; ×445.

FIG. 6.—*Pinus Strobus:* radial section of secondary wood of root close to primary xylem, showing primitive "rims" of Sanio; ×445.

THE DISTRIBUTION OF BEACH PLANTS

EDITH A. ROBERTS

The following observations were made of the distribution of plants on the lower and middle beaches of the coasts of the Elizabethan Islands and Falmouth. The Elizabethan Islands and Falmouth are situated in eastern Massachusetts, Falmouth being a part of the mainland, and the islands extending in a chain into the ocean, bordered by Buzzard's Bay on the west and by Vineyard Sound on the east.

The facts here noted were observed while I was collecting material for the investigation of the osmotic pressure of succulent plants in the above-named localities. A more or less definite grouping of the succulents was found, and, when present, certain plants were always in advance, where they would the more often be covered by the salt water. Later investigation will attempt to show whether the different locations on the beaches present more or less difficult situations for maintenance of growth, and, if so, whether there is a greater or more variable osmotic pressure among the forms to account for their being able to occupy more adverse situations. Competition of species cannot account for the presence or absence of forms, for GANONG (**6**) has shown that the ground is not already occupied, therefore the struggle must be with the physical environment.

Thirteen locations were selected as affording types of all shores on the Islands and Falmouth where succulents might be found. These may be divided into sea beach and sea cliff types. The coasts of the sea beach type are readily divided into lower, middle, and upper beaches according to COWLES (**4**). The upper beach in all cases is a low dune, back of which there is always found a pond. The dune at Chappaquoit Point is the highest.

The sea cliffs may be divided into two classes: those at whose base the rise and fall of water varies so little that a zone of *Zostera marina* is present and those at whose base this plant is absent. The sea beach type was found at Chappaquoit Point, Falmouth

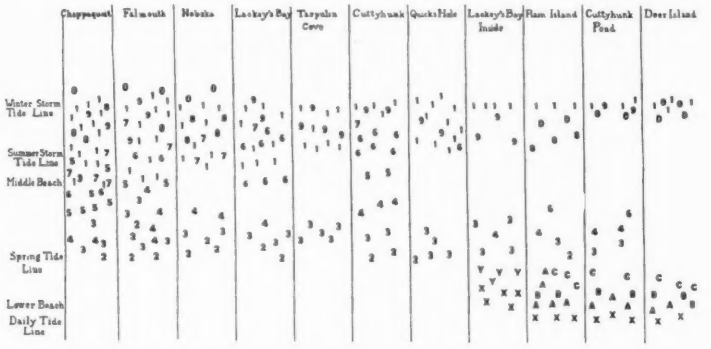
Beach, Nobska Beach, Lackey's Bay, Tarpaulin Cove, Cuttyhunk, and Quicks Hole. The sea cliff without *Zostera marina* was found at Nobska Cliff and Falmouth Cliff. The sea cliff with *Zostera marina* was found at Lackey's Bay (inside), Ram Island, Cuttyhunk Pond, and Deer Island.

The following 9 plants were the characteristic succulents found on the middle beach: *Atriplex arenaria*, *Salsola Kali*, *Chenopodium rubrum*, *Arenaria peploides*, *Cakile edentula*, *Artemisia Stelleriana*, *Solidago sempervirens*, *Euphorbia polygonifolia*, and *Lathyrus maritimus;* while *Ammophila arenaria* has been included in the chart as a tenant of the middle beach because it plays a part in the zonal distribution of some of the succulents. SNOW (**7**) speaks of the upper part of the middle beach as the low *Ammophila* zone, thus subdividing the middle beach into the succulent zone (lower half) and low *Ammophila* (upper half). The drift spoken of as limiting the middle beach at the front is the daily tide line. The spring tide line seems to sweep back the daily tide débris, together with its own gatherings, to a line back of the daily tide. This irregular line of débris at the front of the lower middle beach, determined by the spring tide, marks the extent forward of the succulent zone, while the summer storm tide line marks the beginning of what I term the transitional zone, corresponding, I believe, to the low *Ammophila* zone of SNOW. Succulents and *Ammophila* tenant the region together, with the *Ammophila* dominating just back of the winter storm tide line or on the upper beach, the term *Ammophila* being thus used to apply to the upper beach. The plants exclusive of *Ammophila arenaria* were found in the order given as outpost plants as shown on the chart.

At Chappaquoit Point all the 10 characteristic plants were found. *Atriplex arenaria* was the forerunner, as it always is when present. BERGEN (**1**) states that *Salsola Kali* is the most tolerant of the plants he observed. It ranges here from *Artemisia Stelleriana* to just back of *Atriplex arenaria*, with some plants occasionally at the top of the dune. *Chenopodium rubrum* is confined to a region just back of *Salsola Kali*. *Arenaria peploides* grows in masses and monopolizes a wide zone. *Cakile edentula* grows more in bunches and is closely associated with *Artemisia Stelleriana*,

which often finds its best location at an *Ammophila arenaria* outpost. The *Artemisia Stelleriana* presents more or less of a solid front and only thin strands of *Ammophila* are found beyond; in fact, it might be said to define the border line of upper and middle beaches. *Euphorbia polygonifolia* becomes established on the wind-swept dunes, where there is nothing else, but never is found toward the ocean beyond the limit of *Ammophila* runners. *Lathyrus maritimus* occasionally extends into the transitional zone, but always in company with *Ammophila*.

At Falmouth there is an illustration of what is common at several of the beaches; at the region of the incoming daily tide there is a mass of dry eel grass and immediately back of that a



zone of small shingle, doubtless brought there by the stronger spring tide. At the limit of this shingle a slight depression occurs, then an elevation and the loose sandy zone with the succulents, and beyond this another depression, then an elevation rises slowly to the winter storm tide line.

At Nobska Beach *Chenopodium rubrum* is abundant, with only a little *Atriplex arenaria* and *Salsola Kali*, but wherever all three are present the same relationship holds, *Atriplex* being always nearer the water than the others.

Cakile edentula forms masses in front of *Ammophila* at Lackey's Bay. At Tarpaulin Cove much *Salsola Kali* is found in mats, and bunches of eel grass have been caught by it, forming stable mounds. The beach formed at Cuttyhunk Harbor and Vineyard Sound is

separated from Vineyard Sound by a high shingle mound. Among the shingle *Cakile edentula* grows most luxuriantly and forms thick, hedgelike masses. They are the most abundant and thrifty plants seen. Here the entire shingle strand must be washed often by the summer storms.

The relationship of the plants found at the base of Nobska Cliff and Falmouth is the same as that found on the beaches, but the tide lines are nearer together there, and the zones are condensed. The telescoping comes in the zone between the summer storm tide line and the winter drift, so that there is a more sudden transition from the succulent zone to the *Ammophila* zone, practically eliminating the transitional zone.

The low sea cliffs, at whose base the lower beach is eliminated and where there is living *Zostera marina*, as at Lackey's Bay, furnish an additional grouping. Here, on what corresponds to the plantless lower beach, is a grouping of *Zostera marina* and *Spartina glabra pilosa*. Their advance is checked by a mass of dry eel grass three feet in depth, but on the top of the eel gras, as on the substratum of the lower middle beach, is a grouping of succulent plants with exceptionally long roots. This is a strictly succulent zone, for the *Ammophila* does not advance beyond the eel grass débris. Why succulents can grow on the eel grass substratum and *Ammophila* not is an interesting question.

Along the cliffs where *Zostera marina* is present and the lower beach is covered by water, the following new forms are found: *Suaeda maritima*, *Salicornia europaea*, *Limonium carolinianum*, and *Ligusticum scoticum*. Their ecological relationship is shown on the chart. The last three divisions of the chart show what GANONG (5) speaks of as a *Salicornia-Suaeda* association. CHRYSLER (3) gives a list of plants arranged in a descending scale with respect to salt-resisting capacity, and in this *Salicornia europaea* is next to *Limonium carolinianum*. GANONG found that the root hairs of *Salicornia europaea* can endure 90 per cent of sea water without plasmolysis, and those of *Suaeda maritima* 60–70 per cent. CANNON (2) refers to salt-containing places as having marked zonal distribution; several species of *Atriplex* and *Suaeda* are nearer the center of salt spots, where the concentration of salt is highest.

Summary

1. The plants on the lower and middle beaches of some of the coasts of the Elizabethan Islands and Falmouth are found to have in general definite zonal distribution.

2. The shores may be divided into sea beach and sea cliff types; the latter may be subdivided into cliff types with *Zostera marina* and those without.

3. The following plants were selected as most representative of the beach type: *Ammophila arenaria*, *Atriplex arenaria*, *Salsola Kali*, *Chenopodium rubrum*, *Arenaria peploides*, *Cakile edentula*, *Artemisia Stelleriana*, *Solidago sempervirens*, *Euphorbia polygonifolia*, *Lathyrus maritimus*, and *Suaeda maritima*. *Salicornia europaea*, *Limonium carolinianum*, *Ligusticum scoticum*, *Zostera marina*, and *Spartina glabra pilosa* were taken as representatives of the sea cliff type.

4. The upper half of the middle beach is called the transitional zone.

5. When the lower beach is covered most of the time by water, *Suaeda maritima*, *Salicornia europaea*, and *Limonium carolinianum* are found in the order given, from ocean to shore.

6. The forms of the middle beach are found in definite zones, and when not present their places are not filled; for instance, *Atriplex arenaria*, *Salsola Kali*, *Chenopodium rubrum*, and *Arenaria peploides* are always found in the order given on the lower half of the middle beach, while *Cakile edentula*, *Artemisia Stelleriana*, *Solidago sempervirens*, and *Euphorbia polygonifolia* are related to *Ammophila* in distribution.

7. As to the cause of this distribution I hope to offer some suggestions in the near future.

MOUNT HOLYOKE COLLEGE

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BRIEFER ARTICLES

THE INFLUENCE OF ETHER ON THE GROWTH OF ENDOTHIA

The writers found it necessary to conduct a series of experiments on the influence of ether on the growth of *Endothia*, preliminary to more extensive studies on the influence of the tannin content of the host plant on *Endothia parasitica* and related species. Owing to a change in plans this phase of the work was abandoned. However, it has been considered advisable to publish the results of these experiments.

The medium used was as follows: water 1000 cc., glucose 20 gm., peptone 10 gm., potassium phosphate (dibasic) 0.25 gm., magnesium sulphate 0.25 gm. The organisms used were *Endothia parasitica*, *E. radicalis* (American and European strains), including one from *Quercus alba* which was sent to us by Dr. GEORGE P. CLINTON. This last organism was a much slower grower than the others. In order to prevent the evaporation of the ether, the cotton plugs were pushed a short distance into the tubes and close-fitting corks inserted.

In the first experiments, approximately 10 cc. of the medium was placed in each tube and two or three drops of ether added by means of a graduated pipette (that is, not more than 0.1 cc.). These tubes were then inoculated with the organisms. In all cases the checks grew more slowly than those to which the ether had been added. These results made it necessary to conduct a more careful series of experiments, in which definite but varying amounts of the ether should be used.

In this second experiment, 0.1 cc. ether was added to certain tubes of the medium and 0.4 cc. to others. These were inoculated and kept as above. The results confirmed the first experiments, but made it necessary to conduct a still more extensive experiment.

This experiment was as follows. To 10 cc. of culture solution, ether was added in quantities of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 per cent. In this experiment only one species was used, *E. parasitica*. On the third day the check showed a good normal growth; that having 0.05 per cent ether was somewhat accelerated and that having 0.1 per cent was noticeably so. The next two members of the series showed slight germination. The next day but little change was

noticeable except that germination was apparent up to and including the tubes containing 0.6 per cent ether. The second day the culture containing 0.3, 0.4, and 0.5 cc. ether showed accelerated growth, while the one with 0.6 per cent showed good growth. On the tenth day after inoculation, the cultures with the higher percentages of ether showed signs of more vigorous growth, except the two highest members of the series, which never germinated. From this time on the results do not agree in all details with the earlier stages of the experiment, owing to the unavoidable escape of varying amounts of ether from the different tubes and the consequent change of percentages. Therefore, it was not practicable to keep these cultures containing ether under observation for more than ten days or two weeks, a time too short for pycnidial formation in liquid media.

These results are represented in the accompanying table, in which the check is rated 5, and the acceleration of retardation computed on this basis.

TABLE I

GROWTH OF *Endothia parasitica* IN LIQUID MEDIA TO WHICH THE INDICATED AMOUNTS OF ETHER WERE ADDED (AMOUNT IN CC.)

Days after inoculation	0.0	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
3.....	5	7	9	2	1	0	0	0	0	0	0	0
4.....	5	7	9	4	3	2	1	2	0	0	0	0
5.....	5	5	6	5	4	4	3	3	0	0	0	0
6.....	5	5	6	5	5	5	4	3	0	0	0	0
7.....	5	6	7	6	5	5	4	3	1	0	0	0
8.....	5	6	6	6	5	5	4	3	2	1	0	0
10.....	5	7	6	6	5	5	4	3	2	3	0	0

It appears that small quantities of ether have a stimulating effect on the fungus, quantities of from 0.2 per cent up retard germination, and quantities from 0.4 per cent up have injurious effect on the growth of the fungus. While it is possible to grow the mycelium under the influence of ether, the volatile nature of this chemical makes it impossible to keep such cultures intact long enough for pycnospores to appear. After two to two and one-half weeks the mycelium showed signs of dying and pulling away from the glass in the cultures containing the higher percentages of ether.—MELVILLE T. COOK and GUY W. WILSON, *Rutgers College and New Jersey Agricultural Experiment Station, New Brunswick, N.J.*

WIND BURN IN AMORPHOPHALLUS

Wind burn, or, as it is more commonly called, "sunburn," does not seem to be conspicuous in plants. An interesting case was observed during the past typhoon season at Los Baños, Philippine Islands. During July and August a strong southwest wind is continuous and may often become violent. This wind is frequently warm and dry.

In the latter part of August 1914, the southwest wind was particularly and continuously heavy. By the third day of the heavy wind, large red blotches were noticed on the petioles of plants of *Amorphophallus campanulatus* (Roxb.) Blume (Araceae). Investigation of many plants revealed the fact that only in those plants which were exposed to the wind was the red color present. Light green areas on the petioles were more affected than dark areas. When wind and sun acted in combination, the red color was more apparent, occurring on both blades and petioles. Plants growing in ravines and in places otherwise sheltered from the wind, even though fully exposed to the sun, did not show red coloring. Opening up the brush to allow the wind access resulted in the development of the red coloring within two to three days.

The red color from this cause, however, was not a permanent effect, for in the rainy period which succeeded the heavy wind the red color entirely disappeared within ten days.—FRANK C. GATES, *Los Baños, P.I.*

CURRENT LITERATURE

NOTES FOR STUDENTS

General biology of rusts.—KLEBAHN[1] has published a series of observations on rust fungi made during 1912 and 1913. The report covers three phases of work: a study of the factors which bring about the termination of the rest period in teleutospores, cultural work, and some observations on the mallow rust in relation to ERIKSSON's mycoplasma theory.

In order to study the factors influencing the termination of the rest period in teleutospores, the author subjected teleutosporic material of *Puccinia graminis*, *P. Phragmites*, and *P. Magnusiana* to various treatments such as exposure to low temperatures, alternate wetting and drying, and continuous immersion in water. Apparently the changes in the spore which make germination possible depend not so much upon exposure to low temperatures as upon the action of moisture. Spores suspended in a dry shed and subject only to atmospheric moisture failed to germinate. Alternate wetting and drying is most potent in shortening the rest period; spores subjected to such treatment, even at room temperature, germinated in January two to three months before the usual time. Even continuous immersions with frequent changes of water somewhat shortens the rest period, although occasional drying seems to be essential to produce any marked effect.

The second part of the paper has already been noted.[2] In the third section some experiments and observations on the mallow rust furnish a basis for discussion of ERIKSSON's views on the means of distribution of this fungus. ERICKSSON's main contention[3] is that the fungus is distributed in the mycoplasma state with the seed, and manifests itself by a general outbreak over the entire surface of the infected plant when it is about three months old. These contentions are not borne out by the experiments of KLEBAHN, who finds that different groups of plants grown from the same lot of seed become infected at different times, and the outbreak does not involve the whole plant at once. The sori are at first scattered and few, as if originating from local infections. To the conidia-like segments sometimes produced by the promycelium of this rust ERIKSSON attributes the special function of giving rise to the mycoplasma

[1] KLEBAHN, H., Kulturversuche mit Rostpilzen. Zeitschr. Pflanzenkrank **24**:1–32. *fig. 1.* 1914; also Beobachtungen über Pleophagie und über Teleutosporenkeimung bei Rostpilzen. Jahresb. Verein Angew. Botanik **11**:55–59. 1913 (a short statement of the main facts in the foregoing paper).

[2] Rev. BOT. GAZ. **60**:245. 1915.

[3] *Ibid.* **54**:431. 1912.

in the plants infected by these spores. DIETEL[4] has already shown that the production of "conidia" by the promycelium does not indicate a functional differentiation of the teleutospores, but is dependent on external conditions. In this matter KLEBAHN agrees with DIETEL, but the two investigators differ somewhat as to the factors which determine the segmentation of the germ tubes. KLEBAHN finds that spores germinating within a liquid medium produce, in place of normal mycelia, germ tubes which may become segmented or not. The number of segments produced is always four, and under some conditions the "conidia" produce sporidia. Teleutospores germinating in contact with air always produce promycelia and sporidia. DIETEL attributes the production of segmented germ tubes to a scanty moisture supply in the substratum. KLEBAHN infected a number of leaves of hollyhock with cultures of teleutospores which were producing "conidia." The groups of sori originating from these infections were cut out of the leaves which were kept under observation during the rest of the season. According to ERIKSSON, these leaves should have contained mycoplasma which should later have given rise to a general outbreak of rust on the leaves. KLEBAHN found, however, that the leaves from which the sori had been removed remained free from infection.

The mycoplasma theory is attacked from another standpoint by HAASE-BESSEL,[5] who finds in and about mature rust pustules of secondary origin on leaves of *Althaea rosea* attacked by mallow rust mycelial structures resembling the promycelium described by ERIKSSON. These structures on account of their delicate walls have the appearance of naked masses of protoplasm which conform to the shape of the intercellular spaces which they occupy. A study of their origin shows that they are the outgrowths of older hyphae at the base of the rust pustules. The author attributes to them the function of distributing the mycelium through the leaf. Since these structures, conforming in every way to ERIKSSON's promycelia, are observed originating from the mycelium of mature pustules of undoubted secondary origin, the author argues that the similar structures of ERIKSSON may be explained without the aid of the mycoplasma theory. To this conclusion ERIKSSON would object, as to the other similar arguments, that the mycoplasma does not occur in connection with rust infections originating from spores. Thus the elusive mycoplasma again escapes annihilation.

A cytological study supplemented by field observations has enabled OLIVE[6] to explain some apparently anomalous phenomena in the sequence of spore forms and the formation of aecidia in certain rusts with perennial mycelia.

[4] Rev. BOT. GAZ. **56**:163. 1913.

[5] HAASE-BESSEL, G., Zur Erikssonchen Mycoplasmatheorie. Ber. Deutsch. Bot. Gesells. **32**:393–403. *pl. 9.* 1914.

[6] OLIVE, E. W., Intermingling of perennial sporophytic and gametophytic generations in *Puccinia Podophylli*, *P. obtegens*, and *Uromyces Glycyrrhizae*. Ann. Mycol. **11**:298–311. *pl. 1.* 1913.

The forms investigated are *Puccinia Podophylli*, *P. suaveolens*, and *Uromyces Glycyrrhizae*. Early in spring *Puccinia Podophylli* usually produces teleutospores on the bud sheaths at the base of the stems of the host plant (*Podophyllum peltatum*). These teleutospores are produced before any other spore forms and also before the plants are fully developed. Sometimes, according to OLIVE, traces of aecidia also, but no spermagonia, are found on the bud sheaths. Later, spermagonia and aecidia develop abundantly on the leaves. These aecidia have been shown by SHARP[7] to arise from a binucleate mycelium. Teleutospores are produced on the leaves in late summer. OLIVE finds that the teleutospores on the bud sheaths and the aecidia, both here and on the leaves, arise from a perennial sporophytic (binucleate) mycelium which extends throughout the plant. Intermingled with the sporophytic mycelium is a perennial gametophytic (uninucleate) mycelium which gives rise to spermagonia and to incepts of aecidia which, however, are soon invaded by the sporophytic mycelium whose hyphae give rise to basal cells and rows of binucleate aecidiospores. The mycelia of these two generations are generally commingled throughout the plant, but in the young parts the gametophytic mycelium is somewhat in advance of the sporophytic. Besides these perennial forms, local binucleate mycelia, originating from aecidiospores and giving rise to teleutospores, occur on the leaves. In *Puccinia suaveolens* on the Canada thistle and in *Uromyces Glycyrrhizae* on *Glycyrrhiza lepidota* three conditions of mycelial distribution have been observed by the author: first, perennial gametophytic mycelium giving rise to spermagonia and perennial sporophytic mycelium giving rise to secondary uredospores and to teleutospores are commingled throughout the same plant; second, perennial sporophytic mycelium giving rise to secondary uredospores and to teleutospores occurs alone; third, annual local mycelium occurs which also produces uredospores and teleutospores, but the sori in which these are produced are rarely confluent like those of the perennial mycelium. Perennial gametophytic mycelium alone or local colonies of gametophytic mycelium have not been observed.

In all these cases no spore forms resulting from gametophytic cell fusions have been found. The aecidiospores of *Puccinia Podophylli* and the uredospores of *P. suaveolens* and *Uromyces Glycyrrhizae* are all regarded as secondary in nature. They arise apogamously, solely from sporophytic mycelia. The author has not observed aecidiospores nor primary uredospores arising as the result of fusions between gametes in these forms.

DAWSON[8] has studied the distribution of the mycelium of *Puccinia fusca* and *Aecidium leucospermum* in the rhizomes, buds, and leaves of *Anemone nemorosa*. The mycelium of these fungi is found in the rhizomes and buds of the

[7] SHARP, L. W., Nuclear phenomena in *Puccinia Podophylli*. BOT. GAZ. **51**:463–464. 1911.

[8] DAWSON, W. J., Über das Mycel des *Aecidium leucospermum* und der *Puccinia fusca*. Zeitschr. Pflanzenkrank. **23**:129–137. 1913.

infected plants, but its distribution may be more or less local. Frequently one section of the rhizome with its buds is infected, while the adjacent sections remain free from the fungus. All the leaves arising from the infected sections become infected. Experiments like those reported by TISCHLER[9] for *Uromyces Pisi*, to determine if the plants could outgrow the fungus, were not conducted. The mycelium is found in all the tissues except the phloem and xylem; in the buds even the cells of the dermatogen are invaded. In this respect the distribution of the mycelium in the growing points agrees with that of *Uromyces Pisi* in *Euphorbia Cyparissias* as observed by DE BARY and TISCHLER. There is apparently a marked difference, however, in the behavior of the mycelium of these two fungi in the embryonic tissue. TISCHLER observed the interesting fact that no haustoria were formed by the hyphae of *U. Pisi* in the strictly meristematic cells, while DAWSON states that haustoria are formed in all parts of the plant containing mycelium, even "close" to the growing point. Unfortunately neither the description nor the imperfect sketches make this physiologically important point entirely clear.

TISCHLER[9] has published a further contribution to his interesting studies on the relation between *Uromyces Pisi* and its aecidial host *Euphorbia Cyparissias*. In his former account the fact was brought out that under certain conditions the upper portions of infected shoots of this plant might produce normal leaves to all appearances free from the fungus. In such cases it was found that the growing point had became emancipated from the fungus, which was thereafter unable again to invade the strictly embryonic tissues. The behavior of these shoots is now contrasted with that of shoots from dormant buds of infected rhizomes. Such shoots produce only typically deformed leaves characteristic of plants infected by the fungus. If, however, the infected rhizomes are kept permanently in a vegetative state by pruning or other means, the new shoots produced show no external signs of the fungus and bear only normal leaves. If infected plants, which as a result of such treatment have produced only normal leaves for a season, are allowed to remain dormant for a time, only infected shoots grow out from the dormant buds. It appears, therefore, that buds that develop on the rhizome while the plant is kept continually in a vegetative state, and which start into growth without having undergone a distinct period of rest, are not subject to the formative influence of the fungus; but buds which have undergone a period of dormancy become subject to such influence and produce only deformed leaves. Nevertheless, the apparently sound shoots are not free from the fungus, for the mycelium is found in all parts except the meristematic tissue of the growing point; this it is entirely unable to invade in shoots which have once become emancipated.

[9] Rev. BOT. GAZ. **56**:161. 1913.

[10] TISCHLER, G., Über latente Krankheitsphasen nach *Uromyces*-Infektion bei *Euphorbia Cyparissias*. Bot. Jahrb. **50**:95–110. *figs. 6.* 1914.

Wherein lie the causes of this peculiar difference of behavior of the two kinds of buds the author was unable thus far to determine.

HAAK[11] has published a somewhat lengthy account of a series of general observations and experiments on the biology of *Peridermium Pini*, and particularly on the question of the propagation of this rust by aecidiospores. From the observation that no probable telial host is coextensive in its distribution with *Pinus sylvestris* and its blister rust (*Peridermium Pini*), and also because of the failure up to this time to find the telial phase of this rust, HAAK infers the likelihood that this fungus is propagated on the pine by aecidiospores. This assumption contains in itself nothing anomalous, since several rusts with repeated aecidial generations are known. The experiments by which it is endeavored to substantiate this assumption furnish only probable evidence. Cultures on sound trees having failed to give satisfactory results (out of 72 trees with about 200 sowings only one infection resulted), subsequent cultures were made only on trees which showed the presence of *Peridermium*. The diseased branches were removed and numerous small twigs were infected in wounds. Although the number of *Peridermium* cankers resulting from these infections was small compared with the number of infected twigs, the proportion of diseased twigs to sound twigs was much greater on the infected branches than on the uninfected ones. The circumstantial evidence, therefore, seems to indicate that this fungus can be propagated on the pine by means of aecidiospores. A histological examination to determine whether the mycelium under the aecidia was sporophytic or gametophytic was not made. Two further points of interest in the biology of this fungus are brought out by the author. First, natural infections take place only in green twigs still bearing needles; no infections occur in older twigs. Even old cankers on trunks and large branches, where the origin has not been completely obliterated, can be traced to infections of small twigs from which the disease has gradually spread. Secondly, when a tree is once infected, the disease spreads by new infections to other branches during succeeding years. This fact is in accord with the theory that the aecidiospores are capable of reinfecting the pine.

BAUDYS[12] adds a contribution to the question of the maintainance of rusts by means of wintering uredospores or mycelium. The observations were made during the winters of 1910–1911 and 1911–1912 in Bohemia, where the temperature ranged as low as −16° C. and −21° C. respectively during the two seasons. From 60 to 100 per cent of the uredospores in the winter sori of *Puccinia glumarum* and *P. dispersa* on rye, and *P. bromina* on *Bromus sterilis* were found to retain their capacity for germination throughout the winter. These rusts

[11] HAAK, ———., Der Keinzopf *Peridermium Pini* (Willd.) Kleb. Zeitschr. Forst u. Jagdwesen **46**:3–46. *pls. 2.* 1914.

[12] BAUDYS, E., Ein Beitrag zur Überwinterung der Rostpilze durch *Uredo*. Ann. Mycol. **11**:30–43. 1913.

were kept under observation from early autumn until the general outbreak of rust the following spring. It was found also that young undeveloped sori in the leaves continued to develop when temperature conditions were favorable. The mycelium in the neighborhood of the sori was found to be alive. Uredospores of *Puccinia dispersa* retained their germinative power as long as 100 days, although the percentage of viable spores decreased during that time. The uredospores of a number of other rusts were found to be capable of germinating during the winter, but observations on these forms were not carried through the entire season. Uredospores of *Puccinia simplex* on *Hordeum murinum* germinated on December 29; those of *Uromyces Anthyllidis* on *Anthyllis vulneraria* on December 11; those of *U. Ervi* on *Vicia hirsuta* on January 29; and those of *Puccinia Lolii* on *Lolium perenne* on March 5.

STAKMAN[13] has investigated for the conditions of Minnesota some of the problems relating to the specialization of physiological races of the cereal rusts and the resistance of different varieties of wheat to rusts. The results are in general in accord with the observations of other investigators, and serve to emphasize the fact that particular strains of *Puccinia graminis* inhabiting the common cereals are more or less specifically adapted to particular species, but that this adaptation is not entirely rigid. Of all the forms, that on the oat is most strictly specialized. The forms on wheat, barley, and rye migrate more or less readily from one cereal to another. Treatment of the plants with anaesthetics makes them more susceptible to infection. Resistance to rust seems to depend more on the nature of the variety than upon any external factors to which the plant is subjected. Certain phenomena, such as length of the incubation period of the rust within the plant, size of pustules and of spores, seem to be correlated with the degree of resistance of the host. The observations of MARSHALL WARD that in resistant plants the rust mycelium usually killed small infected areas of the leaves and thereby brought about its own destruction was confirmed.

Miss SAHLI[14] has continued the investigations begun by FISCHER[15] on the susceptibility of hybrids and chimaeras of mixed immune and susceptible parentage to the attacks of fungi to which one parent is susceptible. A number of cultures with teleutospores of *Gymnosporangium Sabinae*, *G. clavariaeforme*, *G. confusum*, and *G. tremelloides*, all inhabiting species of *Crataegus*, *Sorbus*, and *Mespilus*, were made on hybrids and chimaeras of these plants. In general, there appears to be no constant relation between the state of immunity of the parents and that of the hybrids or chimaeras. For the details concerning each case, the paper itself should be consulted.—H. HASSELBRING.

[13] STAKMAN, E. C., A study in cereal rusts. Physiological races. Tech. Bull. **138**. Agric. Exp. Sta. Univ. Minn. pp. 56. *pls.* 9. 1914.

[14] SAHLI, GERTRUDE, Die Empfünglichkeit von Pomaceen-Bastarden und Chimären für Gymnosporangien. Mycol. Centralbl. **3**:10–11. 1913.

[15] Rev. BOT. GAZ. **56**:163. 1913.

Germination.—MÜLLER[16] has done a very important piece of work on the breaking of seed and fruit coats in germination. The work deals with four general topics: (1) source of energy for breaking the coats; (2) structural features aiding in freeing the embryo; (3) measurement of pressure exerted against the coats by the growing contents; and (4) pressure required for bursting the coats in germination.

1. The author finds four different types of seeds and fruits as to the source of the pressure for bursting the coats: (*a*) swelling of the seed or fruit contents resulting from water absorption (swelling endosperm of *Ipomaea* and others, swelling embryo of *Chenopodina maritima*); (*b*) growth of endosperm (*Pinus Pinea*, *Ricinus communis*, and several others); (*c*) growth of cotyledons (*Prunus persica* and several other species of *Prunus*, *Juglans regia*, *J. nigra*, *J. cinerea*, and *Corylus avellana*); (*d*) growth of primary root or hypocotyl. In type (*d*) the author recognizes five different groups: (1) radicle borne between the subtending ends of the cotyledons and exerting its pressure upon the coat through these; (2) embryo located centrally in the endosperm and exerting its pressure upon the coat through this tissue (*Rumex* species, *Ruta graveolens*, *Nigella damascena*, *Viola tricolor*, etc.); (3) no nutrient tissue, embryo directly against the coat, hypocotyl in wedge-shaped cavity (*Helianthus annuus*, *Cucurbita Pepo*, etc.); (4) hypocotyl peripherally placed and in a wedge-shaped channel formed by integuments or by integuments and endosperm or cotyledons (*Ervum lens*, *Phaseolus multiflorus*, *Saponaria vaccaria*, *Potomogeton* species, etc.); (5) root or root-bearing portion of cotyledon generally cylindrical, pushing out through hole stopped by plug, lid, or valves (mostly monocots, as Marantaceae, Musaceae, Cannaceae, Phoenicaceae, etc.).

2. Under structural features of fruit or seed coats related to freeing of the embryo, the author discusses five classes: (*a*) free openings through which embryo grows without resistance (*Coix lacrymae*); (*b*) a special hole for exit of the embryo, the hole being stopped by a plug, cap, or valves (*Canna*, *Tradescantia*, *Commelina*, *Sparganium*, etc.); (*c*) a preformed dehiscent line (*Prunus*, *Pinus*, *Juglans*, *Amygdalus*, etc.); (*d*) zones of slight resistance at which coats break (Polygonaceae, some Lepidocaryeae); (*e*) no special mechanism (*Trigonella foenum graecum*, *Ligustrum vulgare*, *Capsicum annuum*, *Allium* species).

3. The pressure exerted against the coats by growing contents were measured for three forms. The growing cotyledon of *Corylus avellana* develops an average maximal outer pressure on the coat of 4759.42 gm., 34.14 gm. per mm.^{2}, or 3.305 atmospheres. The growing endosperms of *Ricinus communis* and *Pinus Pinea* develop a maximal average pressure of 4539.07 gm.

[16] MÜLLER, GOTTFRIED, Beiträge zur Keimungsphysiologieuntersuchungen über die Sprengung der Samen- und Fruchthullen bei der Keimung. Jahrb. Wiss. Bot. 54:529–644. 1915.

and 4106.96 gm., 32.06 and 38 gm. per mm.^{2}, or 3,104 and 3.678 atmospheres respectively.

4. The hard seed and fruit coats of *Corylus* and *Pinus* are greatly weakened at the dehiscent line merely by water absorption. The breaking resistance offered by the soaked coats in these forms is generally one-half to one-sixth that offered by the dry coat. This weakening is not due to the dissolution of materials from the dehiscent line, for the old breaking strength is recovered upon drying. Soil agencies (bacterial, fungal, and acid effects) and enzymes from the living contents play no part in the dehiscence except where cellulose is present, as in *Juglans*. In *Pinus*, if rings of the coat were buried in soil for a period considerably exceeding germination time, the dehiscent zone was considerably weakened by soil agents. In these experiments, however, the agents can attack the dehiscent zone from every side. Water absorption also greatly lowers the breaking strength of brittle coats like *Ricinus*, as well as leathery or skinlike coats, as in *Vicia Faba*. It seems that the breaking strength of a great number of organic substances (*Laminaria* thallus, filter and parchment paper, etc.) falls greatly with water absorption and rises with water loss.

One must doubt the correctness of a fundamental assumption of MÜLLER in determining whether rupture of the coat is due merely to swelling or mainly to growth of the contents. If the bursting occurs at 0° C., MÜLLER pronounces it due to swelling; if only at a considerably higher temperature, to growth. The coats to be broken are gels of various consistency, which vary in viscosity and other physical characters, including no doubt breaking strength, with temperature, H+, OH−, and salt content. Again, the actual force with which the colloidal contents absorb water varies with conditions. We have found[17] cases where the coats are broken by swelling of the embryo and endosperm, but it occurs only at 15° C. or above, unless the coat is weakened by various treatments, when it occurs at 0° C. It is likely that there are cases where the seed or fruit contents exert much greater pressure upon the coats than those reported by MÜLLER (3–4 atmospheres). Indeed, CROCKER and DAVIS[18] showed by a very different method that the swelling embryo of *Alisma Plantago* exerts a pressure of about 100 atmospheres against the coat. Soil and other natural and applied agents play a much greater part in lowering the breaking strength of seed or fruit coats (*Alisma*, *Sagittaria*, *Amaranthus*, etc.) than MÜLLER found for the seeds upon which he worked. It seems odd that MÜLLER uses the micellar theory in his explanation rather than applying the modern colloid conception. There is no doubt that this leads him to certain incorrect inferences, such as cited above. It is very doubtful whether any generalizations can be drawn from MÜLLER's measurements, since they cover so few forms and in the main the same types of seeds and fruits. For this

[17] Unpublished work by DAVIS and CROCKER.

[18] BOT. GAZ. **58**:285–321. 1914.

reason his pioneer work should be followed by a similar study of a great variety of seeds and fruits.—WILLIAM CROCKER.

Light and growth.—VOGT[19] has shown that the effect of light upon the growth rate of the coleoptile of *Avena sativa* is very complex, in contrast to the older statement that light always inhibits growth. Temporary illumination of sufficient intensity is shortly followed by a considerable decrease, which in turn is immediately followed by a greater and longer enduring increase in growth rate. This increase is due to the action of light alone, and not to the combined action of light and darkness. The initial inhibitory effect of light on growth is not due to increased transpiration, as BLAAUW[20] suggests. In sufficient illumination the rate and total amount of growth of this organ is reduced. The greater amount of illumination causes a second inhibitory effect, which greatly exceeds the former acceleration, so that the total effect is a marked reduction in rate and amount of growth. This total effect of light was all that was definitely studied before JACOBI'S[20] work in 1911. In this total effect, like amount of light gives like effect, even within wide variation of the intensity and duration factors. For a given reduction in growth, *light intensity*×*duration of illumination* is a constant. This is the quantity of stimulus law which has been shown to have rather general application in growth and movement stimuli in plants. It seems then that there are three effects of light: inhibition, followed by a greater acceleration upon temporary illumination, and a second inhibition largely determining the total effect in long or enduring illumination of sufficient strength. These results tally in general with those of BLAAUW on sporangiophores of *Phycomyces*, except that BLAAUW has not found important the initial inhibitory effect of light.

While all the work of JACOBI, BLAAUW, and VOGT will greatly modify the statements current in our texts, there is one set of experiments by VOGT which is especially interesting in this respect. When the organ was alternately illuminated (100 M.K. or less) and darkened in 15, 30, or 60-minute periods, the illuminated periods gave less growth only in the 30 and 60-minute periods, and not in the 15-minute periods. In the longer periods, VOGT has shown that the greater growth in darkness is due largely to the stimulation effect of the previous illumination, and the slower growth in light is not due in the main to inhibitory effect of light. This quite reverses the former interpretation given to such results.

Red light acts as very weak diffuse light. This tallies with the known existence in the red of photo-stimulation power. A sudden change of 10–12° C. in the temperature has effects very similar to temporary illumination. This leads VOGT to inquire whether the effect of temporary illumination is not due

[19] VOGT, E., Über den Einfluss des Lichts auf das Wachstum der Koleoptile von *Avena sativa*. Zeitschr. Bot. **7**:193–270. 1915.

[20] BOT. GAZ. **59**:67–68. 1915.

to a purely physical change, perhaps a temperature change. Some will object to the distinction between physical and physiological changes, for is not the latter, as well as its physical aspects, largely unknown? In this connection it might be mentioned that we know little about the effect of light upon "hydration" of colloids in general, and of cell colloids especially. Recently we have been coming to see that this is quite as important in growth as is turgor pressure.—WILLIAM CROCKER.

Embryo sac of Myosurus.—In 1913 NAWASCHIN and FINN[21] concluded that chalazogams are primitive and derived from gymnosperms, the conclusion being based largely upon the reduction series shown by sperms. They found that the generative cell of *Juglans* reaches the embryo sac as a binucleate cell, which means that nuclear division had occurred, but that distinct male cells had not been organized. This condition, carried over from gymnosperms, was regarded as an intermediate one between a well developed sperm and a naked sperm nucleus. TCHERNOYAROW[22] has now found the same condition in *Myosurus minimus*, and concludes that this is probably a feature of the "Polycarpicae," and that this phylum and the chalazogams are two independent primitive branches from the gymnosperm stock. The paper also contains a detailed account of the events from the development of the pollen tube to the act of fertilization. In general the events are of the usual kind, but attention may be called to the fact that the author lays special stress upon the idea that there is some coordination which assures the discharge of the pollen tube at the moment of maturity of the embryo sac.—J. M. C.

Northern plains forests of Canada.—A recent report by CONNELL[23] deals with the forest region lying north of the prairies in Manitoba, Saskatchewan, and Alberta. It extends from the contact line with the Laurentian pine plain near Lake Winnipeg in the east, to the Rocky Mountains in the west. The northern limits are not determined. If the portion of northern Alberta most carefully studied be taken as typical of the entire region, it is made up as follows: (1) boulder clay slopes, which comprise about 50 per cent of the area, more than half of these slopes being covered with a poplar association, a little less than one-fourth with a mixed spruce-poplar forest, and less than 2 per cent with a pure spruce association; (2) sand ridges, which amount to 18 per cent of the area and are covered with nearly pure *Pinus Banksiana*, *P. Murrayana* appearing in mixture with it in the western portion of the region; (3) swamps, which occupy the remainder, showing how poorly developed the drainage is. In the swamps there is a stunted growth of *Picea mariana* and *Larix laricina*, the former predominating.—GEO. D. FULLER.

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[21] Rev. BOT. GAZ. **57**:162. 1914.

[22] TCHERNOYAROW, M., Les nouvelles données dans l'embryologie du *Myosurus minimus* L. Mém. Soc. Nat. Kiew **24**:95–170. 1915.

[23] CONNELL, A. B., Some aspects of the nothern plains forest region of Canada. Forestry Quarterly **13**:31–34. 1915.